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ORIGINAL ARTICLES

THE EFFECTS OF NISENTIL HCl (ALPHAPRODINE HCl) AND LEVALLORPHAN TARTRATE ON RESPIRATION

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It has been shown recently by Siker *et al.*[11] that Nisentil HCl (1,3-dimethyl-4-phenyl-4-propionoxy-piperidine hydrochloride)*, a short-acting analgesic, can be used with advantage to supplement nitrous oxide-oxygen-thiopental sodium anesthesia. These workers observed that the mg./minute dose of thiopental sodium was greatly reduced and the percentage of patients who reacted to stimulation at the end of the operation significantly increased if alphaprodine, rather than meperidine, was used for supplementation. The most frequently encountered side effect of alphaprodine, when given in unduly large doses, was a marked decrease in respiratory rate and a less marked, but definite, diminution in tidal volume. Therefore, investigation of the efficacy of a narcotic antagonist against this respiratory depression seemed advisable.

Several narcotic antagonists have been described since Pohl[10] experimented with N-allylnorcodeine. Of these, N-allylnormorphine has received widest attention. This drug was first used clinically by Eckenhoff *et al.*[3], and has since been investigated by Eckenhoff *et al.*[4], Landmesser *et al.*[8], Bodman[2] and other workers. A recently introduced antagonist, levo-3-hydroxy-N-allylmorphinan tartrate**, showed

*The generic name of Nisentil HCl is alphaprodine hydrochloride. For brevity the compound will be referred to hereafter as alphaprodine.

**The generic name of levo-3-hydroxy-N-allylmorphinan tartrate is levallorphan tartrate and its experimental number designation, Rol–7700. For brevity the compound will be referred to hereafter as levallorphan or as Rol–7700.

considerable promise in its early trials; this drug was used in the present study.

Fromherz and Pellmont[5] reported that, in animals, levallorphan neutralizes the respiratory effects of morphine and levo-Dromoran tartrate. Similarly, Benson *et al.*[1] showed, in rabbits, that narcotic-induced depression of respiratory rate and volume is reversed by levallorphan. The clinical use of levallorphan was first reported by Hamilton and Cullen[7]. They gave 14 patients nitrous oxide-oxygen anesthesia supplemented by deliberate overdoses of intravenously administered levo-Dromoran tartrate, meperidine or morphine. They reported that the action of the antagonist was apparent within one minute; that, after an initial peak effect, there was some decrease in the antagonist's action, but that over a period of 4 hours respiration remained well above the previously depressed levels. In 5 patients, who were given levallorphan prior to the narcotic, respiratory depression did not occur.

Material and Method. The present study comprises a total of 210 conscious subjects°, whose ages ranged from 16 to 81 years (average 47 years) and who underwent surgery under subarachnoid or epidural block, not extending above the level of T 12. Their weights ranged from 47 to 115 kg. (average 73.2 kg.). The investigation was carried out in two parts. In the first part the effects of alphaprodine and levallorphan on respiratory rates of 120 patients who breathed room air were investigated. The second part was a kymographic study of the changes in respiratory rate, tidal volume, minute volume, and alveolar ventilation rate, which followed the administration of these drugs to 90 patients who breathed 100% oxygen. No patient was tested on more than one occasion.

All 210 subjects were premedicated with 50 to 100 mg. of pentobarbital sodium, given orally 90 to 120 minutes preoperatively and with a combination of 5 to 10 mg. of morphine sulfate and 0.3 to 0.4 mg. of scopolamine hydrobromide, administered subcutaneously 45 to 60 minutes before the regional block. Time was allowed for stabilization of pulse rate, blood pressure and respiration before the tests were performed.

Preliminary trials showed that 1 mg./kg. of alphaprodine consistently produced apnea or gross respiratory depression in these premedicated patients. This dose of alphaprodine was used generally, except in a few aged or debilitated subjects in whom a dose of 0.75 mg./kg. was employed. The solutions of alphaprodine°° (15 mg./cc.) and levallorphan°° (1 mg./cc.) were administered intravenously. Results were expressed in per cent of control values.

PART I. EFFECTS OF ALPHAPRODINE AND LEVALLORPHAN ON RESPIRATORY RATE IN PATIENTS BREATHING ROOM AIR. *Experimental Procedure.* The 120 patients studied were divided into three groups of 40 subjects, each of whom received: a) alphaprodine followed by levallorphan at the time of maximal respiratory depression°°° b) alphaprodine and levallorphan combined in a single injection, or c) levallorphan followed after four minutes by alphaprodine.

Levallorphan was given at two different dose levels. Thus, in each of the three groups half of the patients received 0.01 mg./kg. and the other half 0.02 mg./kg. of the antagonist. These doses correspond to levallorphan: alphaprodine ratios of 1:100 and 1:50. Alphaprodine was administered in 30 seconds; levallorphan was injected as rapidly as feasible through a 25 gauge needle.

Respiratory rate, blood pressure and pulse rate were recorded before the tests and at the intervals indicated in Sections A–C of Fig. 1. Observations of the state of consciousness of the patients and of the occurrence of side effects were also made. To prevent undue hypoxia, oxygen was administered by face mask whenever necessary.

Results. a) ALPHAPRODINE FOLLOWED BY LEVALLORPHAN. Section A of Fig. 1 shows the mean respiratory rates of the two subgroups of 20 patients each, at the alphaprodine-induced nadir of

°Three additional patients were excluded from the study for reasons to be discussed later (see page 246).

°°Nisentil HCl and levallorphan tartrate were kindly supplied by Hoffmann-La Roche Inc., Nutley, New Jersey.

°°°The nadir of respiratory depression occurred 2 to 3½ minutes after administration of alphaprodine.

respiratory rate and at fixed intervals after administration of the two dose levels of levallorphan given at the time of maximal respiratory depression. As is seen, alphaprodine produced a marked decrease of the respiratory rate, levallorphan caused considerable reversal of this depression, and the two dose levels of the antagonist were about equally effective.

Alphaprodine caused sleep in 26 and drowsiness in 14 of the 40 patients. Two subjects had marked diaphoresis shortly after injection of alphaprodine. In one case whealing developed along the injected vein.

b) ALPHAPRODINE AND LEVALLORPHAN COMBINED IN A SINGLE INJECTION. Section B of Fig. 1 shows the mean respiratory rates of the two subgroups of 20 patients each, at fixed intervals after injection of alphaprodine combined with levallorphan at the two dose levels. It will be noted that the addition of levallorphan prevented the alphaprodine-induced respiratory depression. There was no difference between the effects of the two doses of levallorphan.

Twenty-nine patients went to sleep following administration of the drug mixtures. The remainder showed various degrees of drowsiness. Four patients complained of nausea. Marked sweating was observed in 3 cases. Four of the 20 subjects in each of the two subgroups required oxygen.

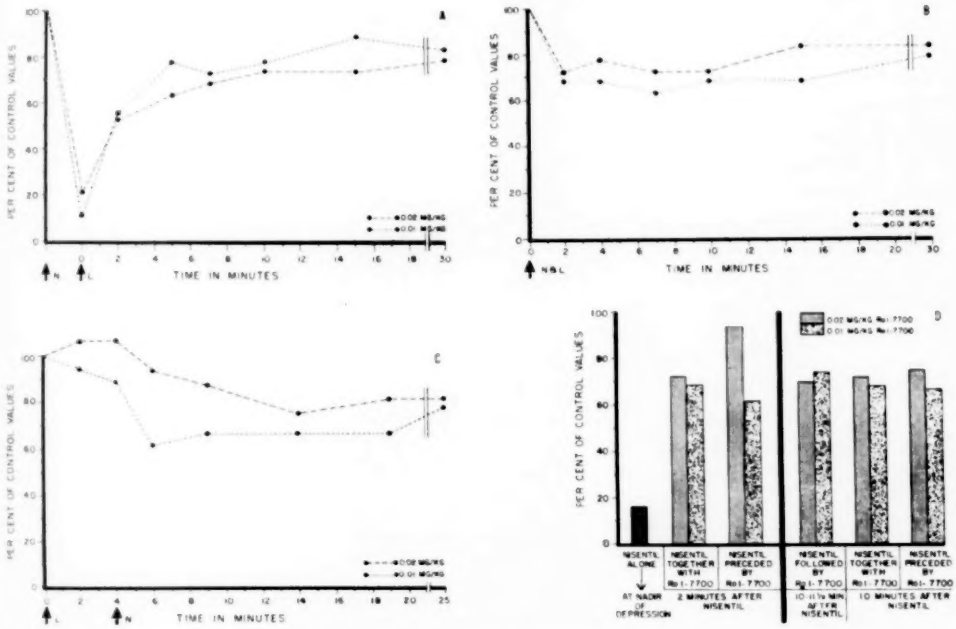

Fig. 1.—Average changes in respiratory rates of 120 patients breathing room air who were given 1 mg./kg. of Nisentil (N) and levallorphan (L), as indicated. *A*) Findings with N followed at maximal depression of respiratory rate (2 to 3½ minutes after N) by L (20 patients each dose). *B*) Findings with N combined with L (20 patients each dose). *C*) Findings with L (20 patients each dose) followed after 4 minutes by N. *D*) Comparison of effects on respiratory rates at the times indicated after N. Bar after N alone represents average of observations for 40 patients. All other bars represent averages for 20 patients. (Rol–7700 = L).

c) LEVALLORPHAN FOLLOWED BY ALPHAPRODINE. Section C of Fig. 1 shows the mean respiratory rates of the two subgroups of 20 patients each, after administration of the two dose levels of levallorphan at fixed intervals after injection of alphaprodine. It should be noted that neither dose level of levallorphan had any significant effect on respiratory rates. The subsequent administration of alphaprodine, however, caused some decrease of these rates. Again there was little difference between the effects of the two dose levels of levallorphan. Both doses of the antidote prevented the extreme respiratory depression which followed alphaprodine alone.

No drowsiness was produced by levallorphan. Twenty-eight patients were asleep shortly after they had received alphaprodine; the remainder exhibited varying degrees of drowsiness. Nausea and retching occurred in one patient. Seven of the subjects who had received the smaller and four of those who had received the larger dose of the antidote, had to be given oxygen for impaired ventilation following the administration of alphaprodine.

In the above three subgroups alphaprodine, levallorphan, or these drugs combined did not produce any consistent changes in pulse rate or blood pressure.

PART 2. KYMOGRAPHIC RESPIRATORY STUDIES OF EFFECTS OF ALPHAPRODINE AND LEVALLORPHAN ON RESPIRATORY RATE, TIDAL VOLUME, MINUTE VOLUME AND ALVEOLAR VENTILATION RATE IN PATIENTS BREATHING OXYGEN. *Experimental Procedure.* The kymographic tracings for the 90 patients included in this part were made with a Sanborn basal metabolism apparatus (Metabolizer), equipped with a CO_2 absorber, which had been previously calibrated with the flowmeter of a Forreger anesthesia machine.

After regional anesthesia had been established and the patients had become stabilized, the mask of the Metabolizer was strapped to their faces and they were allowed to accustom themselves to breathing through the apparatus. Every effort was made to obtain an airtight fit.

When the respirations had become fairly regular, the tests were initiated. Control values of respiratory rates, tidal volumes, and minute volumes were obtained before drug administration.

The patients were divided into three groups of 30 subjects, each of whom received: a) alphaprodine followed 2 minutes later by levallorphan (not at the time of maximal respiratory depression as in the previous series), b) alphaprodine and levallorphan combined in a single injection, or c) levallorphan followed 4 minutes later by alphaprodine.

Levallorphan was administered at three different dose levels. Thus, in each of the three groups 10 patients each received 0.01, 0.02, and 0.04 mg./kg. of the antagonist. These doses correspond to levallorphan : alphaprodine ratios of 1 : 100, 1 : 50 and 1 : 25. The speed of injection of alphaprodine and of levallorphan were the same as described in Part 1.

Tracings were continued for 15 minutes after the last drug administration. Almost all patients tolerated the experimental conditions well for this length of time. In the few who objected to the facepiece, the test had to be terminated prematurely.

The respiratory rates were read from the tracings. The tidal volumes, expressed in cc., were determined by measuring the depth of each inspiration in mm. and by multiplying this figure by 29 (the calibration constant of the instrument). Minute volumes were obtained by summation of all tidal volumes within a one-minute period. Alveolar ventilation rates were calculated by subtracting dead space ventilation values (200* × respiratory rate) from minute volumes. Tidal and minute volumes and alveolar ventilation rates were subsequently converted to liters per square meter of body surface. Observations on the consciousness of the patients and on side effects were also made.

Results. EFFECTS ON RESPIRATORY RATES. a) *Alphaprodine followed by levallorphan.* Figure 2A shows the mean effects on respiratory rates of alphaprodine and of the three dose

*This figure includes physiological dead space plus an estimated 50 cc. for the dead space of the mask.

levels of levallorphan in the three subgroups of 10 patients each, who received the antagonist 2 minutes after the narcotic. Alphaprodine decreased respiratory rates in all three groups, but there was a considerable scatter in the degree of depression. Levallorphan effects of the 0.02 and 0.01 mg./kg. doses were statistically significant only at 10 minutes after administration of levallorphan (see Table 1).

b) *Alphaprodine and levallorphan combined in a single injection.* Figure 2B depicts the changes in the mean

TABLE 1.—STATISTICAL SIGNIFICANCE OF THE DIFFERENCES OBSERVED IN RESPIRATORY RATES AND MINUTE VOLUMES WITH THE THREE DOSE LEVELS OF LEVALLORPHAN IN PATIENTS BREATHING OXYGEN

Sequence of Drug Administration	*Time in Minutes Following the Administration of:*		*t* Values for the % Changes of Respiratory Rates*			*t* Values for the % Changes of Minute Volumes*		
	Nisentil	*Levallorphan*	t_1	t_2	t_3	t_1	t_2	t_3
Nisentil Followed by Levallorphan After 2 minutes								
	4	2	3.4	2.7	NS	7.1	5.0	2.5
	6	4	3.1	2.8	NS	4.0	4.3	NS
	7	5	2.6	2.1	NS	3.2	4.1	NS
	9	7	5.1	2.5	NS	5.1	3.2	2.3
	12	10	4.4	1.8	2.5	5.2	4.4	NS
Nisentil and Levallorphan Combined in Single Injection	2	2	NS	NS	NS	NS	NS	NS
	4	4	2.7	1.9	NS	2.0	NS	2.3
	5	5	2.9	2.1	NS	NS	NS	NS
	7	7	2.4	2.2	NS	NS	NS	NS
	10	10	1.8	NS	NS	NS	NS	NS
	15	15	NS	NS	NS	NS	NS	NS
Levallorphan Followed by Nisentil After 4 minutes	–	2	NS	NS	NS	NS	NS	NS
	0	4	NS	NS	NS	NS	NS	NS
	2	6	NS	NS	NS	NS	NS	NS
	4	8	2.5	NS	2.1	NS	NS	NS
	5	9	2.0	NS	1.8	2.0	NS	NS
	7	11	3.7	2.2	NS	NS	NS	NS
	10	14	2.2	3.0	NS	NS	NS	NS
	15	19	NS	NS	NS	NS	NS	NS

*t_1, t_2, and t_3 signify the differences between the 0.04 and 0.01, 0.04 and 0.02, and 0.02 and 0.01 mg./kg. doses of levallorphan, respectively.
NS = Not Significant.

increased the respiratory rate in proportion to the size of the dose. There was at all times a statistically significant difference between the effects of the 0.04 mg./kg. dose and the two other dose levels of the antagonist. However, the differences between the respiratory rates in the three subgroups of 10 patients each, after administration of alphaprodine combined with levallorphan at the three dose levels. There was some decrease in respiratory rates in all three subgroups, and the least depression occurred in the 10 pa-

tients who received the mixture of alphaprodine and levallorphan which contained 0.04 mg./kg. of the latter drug. There was a statistically significant difference between the effects of the 0.04 and 0.01 mg./kg. doses of the antagonist at 4, 5, 7 and 10 minutes after drug administration. The difference between the effects of the 0.04 and 0.02 mg./kg. doses of the antidote was significant at 4, 5 and 7 minutes after administration of the mixtures. There was no significant difference between the effects of the 0.01 and 0.02 mg./kg. doses (see Table 1).

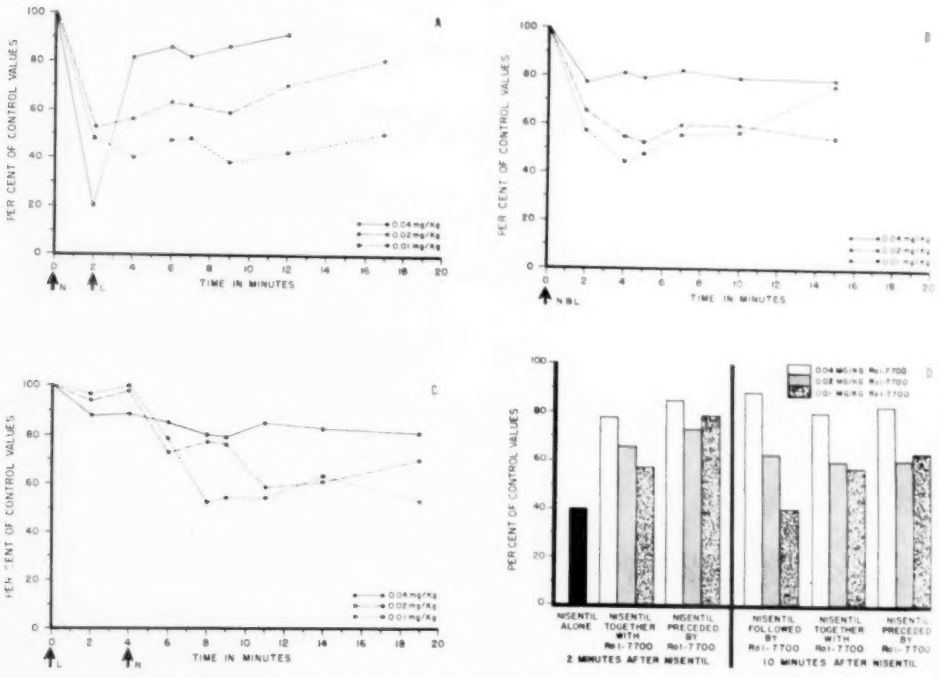

Fig. 2.—Average changes in respiratory rates of 90 patients breathing oxygen, who were given 1 mg./kg. of Nisentil (N) and levallorphan (L), as indicated. *A*) Findings with N followed after 2 minutes by L (10 patients each dose). *B*) Findings with N combined with L (10 patients each dose). *C*) Findings with L (10 patients each dose) followed after 4 minutes by N. *D*) Comparison of effects on respiratory rates at the times indicated after N. Bar after N alone represents average of observations for 30 patients. All other bars represent averages for 10 patients. (Rol–7700 = L.)

c) *Levallorphan followed by alphaprodine.* Figure 2C portrays the mean protective effects on respiratory rates of the three dose levels of levallorphan given to the three subgroups of 10 patients each, 4 minutes prior to alphaprodine. The antidote itself caused a slight decrease in respiratory rates. This was most marked with the 0.04 mg./kg. dose. After the administration of alphaprodine, there was a further depression of the rates. This appeared to be least marked in patients who had received the largest dose of levallorphan. There was a significant difference between the effects of the 0.04 and 0.01 mg./kg. doses of levallorphan at 8, 9, 11 and 14 minutes after their administration. The difference between

the effects of the 0.04 and 0.02 mg./kg. doses was significant only at 11 and 14 minutes and, between the effects of the 0.01 and 0.02 mg./kg. doses, at 8 and 9 minutes after administration of the antagonist (see Table 1).

minutes after the narcotic. Alphaprodine caused an almost 50% reduction in the tidal volume in all three subgroups and the antidote, at all dose levels, reversed this effect. In fact, the tidal volume was usually increased to

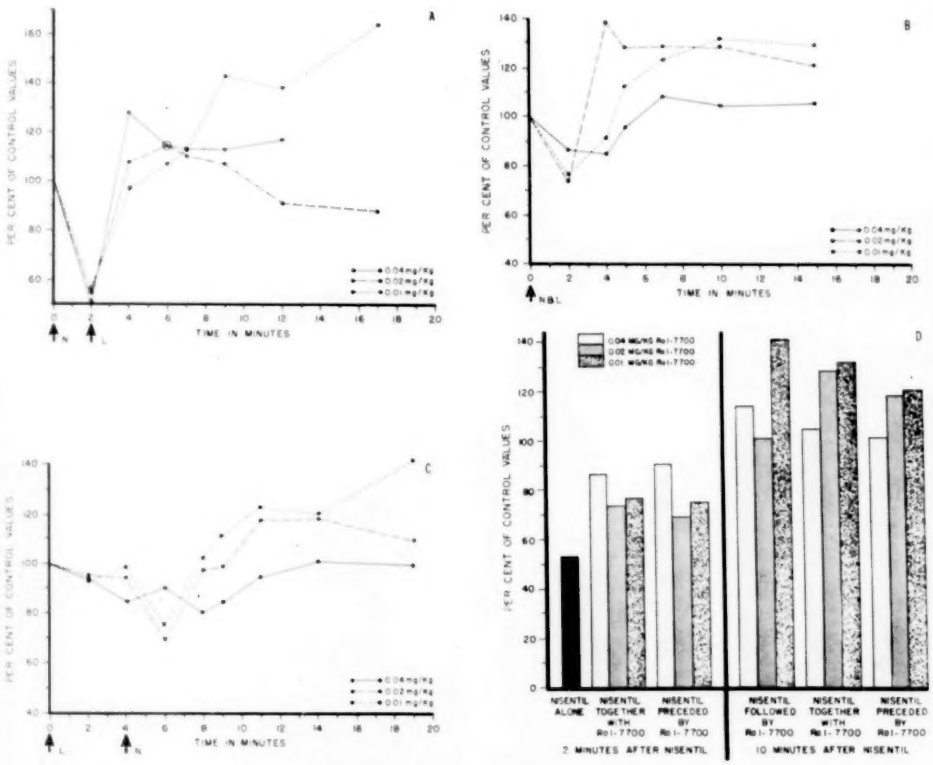

Fig. 3.—Average changes in tidal volumes of 90 patients breathing oxygen, who were given 1 mg./kg. of Nisentil (N) and levallorphan (L), as indicated. *A*) Findings with N followed after 2 minutes by L (10 patients each dose). *B*) Findings with N combined with L (10 patients each dose). *C*) Findings with L (10 patients each dose) followed after 4 minutes by N. *D*) Comparison of effects on tidal volumes at the times indicated after N. Bar after N alone represents average of observations for 30 patients. All other bars represent averages for 10 patients. (Rol–7700 = L.)

EFFECTS ON TIDAL VOLUME. a) *Alphaprodine followed by levallorphan.* Figure 3A shows the mean effects on tidal volume of alphaprodine and of the three dose levels of levallorphan in the three subgroups of 10 patients each, who received the antagonist 2 above 100% of control. There was no significant difference between the effects of the three dose levels of levallorphan.

b) *Alphaprodine and levallorphan combined in a single injection.* Figure 3B depicts the mean changes in tidal

volumes in the three subgroups of 10 patients each, after administration of alphaprodine combined with levallorphan at the three dose levels. The injection of the mixture resulted in a fall of tidal volume, but the lowest level was considerably higher than with alphaprodine alone. There was considerable scatter of the individual results. The tidal volume, however, returned to normal or above within a few minutes. There was no significant difference between the results with the three levallorphan dose levels.

c) *Levallorphan followed by alphaprodine.* Figure 3C portrays the mean protective effects on tidal volumes of the three dose levels of levallorphan given to the three subgroups of 10 patients each, 4 minutes prior to alphaprodine. Levallorphan itself produced a slight fall of tidal volume. The administration of alphaprodine produced a further fall, but this quickly returned to normal or above normal levels. There was no significant difference between the effects of the three dose levels of levallorphan, but the degree of scatter of individual results was great.

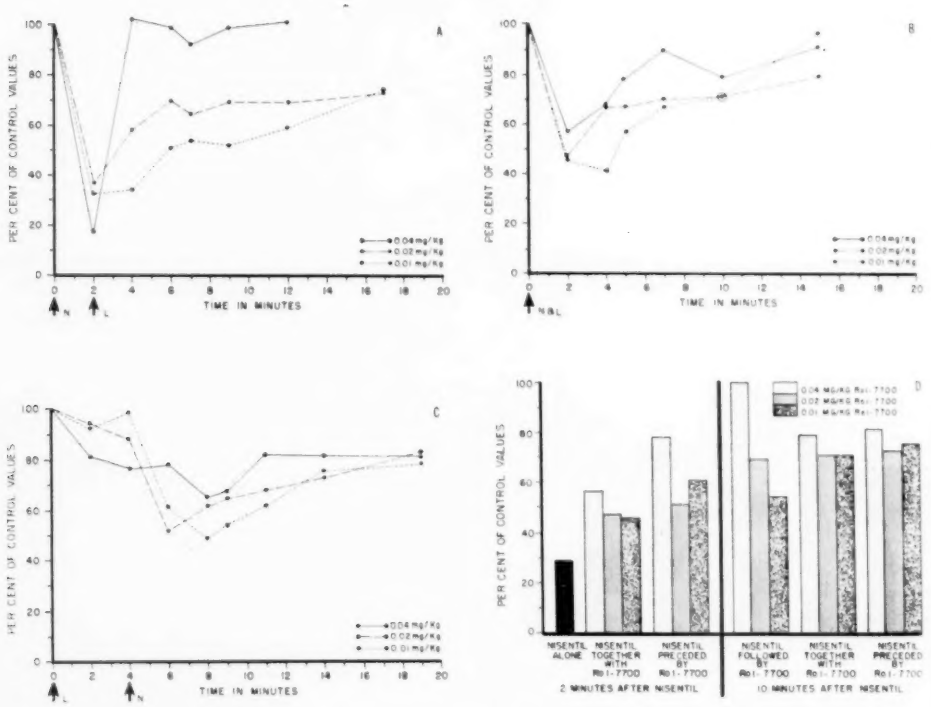

Fig. 4—Average changes in minute volumes of 90 patients breathing oxygen, who were given 1 mg./kg. of Nisentil (N) and levallorphan (L), as indicated. *A*) Findings with N followed after 2 minutes by L (10 patients each dose). *B*) Findings with N combined with L (10 patients each dose). *C*) Findings with L (10 patients each dose) followed after 4 minutes by N. *D*) Comparison of effects on minute volumes at the times indicated after N. Bar after N alone represents average of observations for 30 patients. All other bars represent averages for 10 patients. (Rol–7700 = L).

EFFECT ON MINUTE VOLUME. a) *Alphaprodine followed by levallorphan.* Figure 4A shows the mean effects on

minute volume of alphaprodine and of the three dose levels of levallorphan in the three subgroups of 10 patients each, who received the antagonist 2 minutes after the narcotic. It will be noted that alphaprodine decreased the minute volume in all three groups. Two minutes after the injection of the 0.04 mg./kg. dose of the antagonist, the minute volumes returned to normal and remained practically at control values for the duration of the tests. On the other hand, with the other two dose levels of levallorphan, the minute volumes were only about 70% of the control value at termination of the observation period. Thus, at all times the 0.04 mg./kg. dose of levallorphan had a significantly greater effect than the two lower doses. Except for the values obtained at 2 and 7 minutes after levallorphan, there was no significant difference between the effects of the 0.02 and 0.01 mg./kg. doses of the antagonist (see Table 1).

b) *Alphaprodine and levallorphan combined in a single injection.* Figure 4B depicts the mean changes in minute volumes in the three subgroups of 10 patients each, after administration of alphaprodine combined with levallorphan at the three dose levels. As will be noted, the mixtures caused a sharp fall in minute volume. However, this fall was markedly less than that caused by alphaprodine in the 30 patients, who received the narcotic first. There was a considerable recovery by the end of the test period with little difference between the effects of the three dose levels of levallorphan (see Table 1).

c) *Levallorphan followed by alphaprodine.* Figure 4C portrays the mean protective effects on minute volumes of the three dose levels of levallorphan given to the three subgroups of 10 patients each, 4 minutes prior to alphaprodine. Levallorphan caused a moderate fall of minute volumes. The injection of alphaprodine caused further depression of the minute volumes which partly recovered, but were still below control values at the end of the test period. The difference between the effects of the three dose levels of the antidote was not statistically significant (see Table 1).

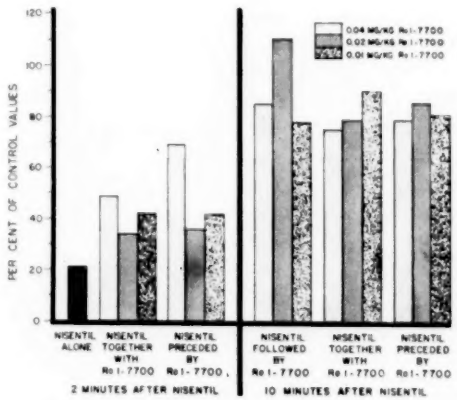

Fig. 5.—Comparison of effects on alveolar ventilation rates of 1 mg./kg. of Nisentil and levallorphan (Rol–7700), at three dose levels, at the times indicated after Nisentil.

EFFECTS ON ALVEOLAR VENTILATION. The left hand portion of Fig. 5 shows that 2 minutes after alphaprodine the

average alveolar ventilation decreased to 21% of the control value (2.05 L/m²). Levallorphan did not provide complete protection against the alphaprodine-induced reduction of alveolar ventilation rate. The least depression occurred in the 10 patients who received 0.04 mg./kg. of levallorphan followed after 4 minutes by alphaprodine. In these instances the average alveolar ventilation rate was 69% of the control value. As appears from the right hand portion of Fig. 5, the mean alveolar ventilation values, 10 minutes after the injection of alphaprodine, ranged from 76 to 112% of the average control value.

SIDE EFFECTS. No significant changes in pulse rate and blood pressure were observed in the 90 patients who breathed oxygen and received alphaprodine and levallorphan as described above. In 3 cases whealing was present along the course of the injected veins. Nausea and retching occurred in 3 patients and these were excluded from the study (see footnote, page 238) since an airtight closure of the system could not be maintained. The percentage of patients, who fell asleep in this group, was approximately the same as in the group which breathed room air.

Discussion. It is apparent from the first part of this study, carried out in patients who breathed room air, that the administration of levallorphan counteracted or prevented to a marked degree the alphaprodine-induced depression of respiratory rate. However, levallorphan, at either dose level, did not cause the respiratory rate to return to normal by the end of the test period, with any of the sequences of drug administration.

The left hand portion of Section D of Fig. 1 shows that there was a marked difference between the mean respiratory rates after the administration of alphaprodine with and without the antagonist. When given together with alphaprodine, results with the two dose levels of the antidote were quite similar. However, when the antagonist was administered before alphaprodine, the 0.02 mg./kg. dose afforded greater protection, 2 minutes after administration of the narcotic.

As is seen from the right hand portion of Section D of Fig. 1, the mean respiratory rates, at approximately 10 minutes after the injection of alphaprodine, were quite similar with all three sequences of drug administration and with both dose levels of levallorphan. The mean respiratory rates at this point ranged from 67 to 74% of control values.

The results in the second part of this study, in patients who breathed oxygen, show that alphaprodine affects alveolar ventilation rate, minute volume, and respiratory rate to a greater degree than the depth of respiration.

The study of the changes of respiratory rates reveals that, regardless of the sequence of drug administration, the 0.04 mg./kg. dose of levallorphan provided the greatest protection against depression of respiratory rates. The injection of the antidote before alphaprodine usually resulted in the least depression of respiratory rate.

The left hand portion of Section D of Fig. 2 shows that there was considerable difference between the mean respiratory rates 2 minutes after alphaprodine, with and without administration of the antagonist. As appears from the right hand portion of Section D of Fig. 2, respiratory rates, at 10 minutes after alphaprodine, averaged 79 to 88% of control values after the 0.04 mg./kg. doses of levallorphan. At this point results with the two lower doses of levallorphan were quite similar, but inferior to those obtained with the 0.04 mg./kg. dose. The only exception was that the 0.01 mg./kg. dose of levallor-

phan given after alphaprodine had no protective effect.

The difference between the degree of depression of respiratory rate caused by alphaprodine, in the patients who breathed room air and those who breathed oxygen, is due to the fact that the rate in the latter was determined 2 minutes after the administration of alphaprodine and in the former at the nadir of depression. It appears that after comparable doses of levallorphan and alphaprodine, the respiratory rates were much less depressed when the patients breathed room air than when they breathed 100% oxygen. This is in line with clinical experience.

The tidal volume, although definitely depressed by alphaprodine, was rapidly increased to normal or above normal levels by the antidote. Although initially there was no significant difference between the effects of the three dose levels of levallorphan, later during the test period, best results were obtained with the 0.01 mg./kg. dose. This may be explained by the fact that a more marked decrease of the respiratory rate, which occurred with the lowest dose of levallorphan, led to a greater accumulation of CO_2 and this in turn increased tidal volume more markedly.

As is seen in the left hand portion of Section D of Fig. 3, the mean tidal volumes, 2 minutes after administration of alphaprodine, were approximately 90% of the control values when 0.04 mg./kg. levallorphan were given together with or before the narcotic. With the two lower dose levels of the antagonist, the corresponding values ranged from 69 to 77% of the control values.

Furthermore, it appears from the right hand portion of Section D of Fig. 3 that the mean tidal volumes, at 10 minutes after the injection of alphaprodine, were highest with the 0.01 mg./kg. dose of the antagonist.

It is obvious that levallorphan, given simultaneously with or preceding alphaprodine, partially prevented the fall of minute volume which followed administration of alphaprodine alone. The 0.04 mg./kg. dose of the antagonist, given 2 minutes after alphaprodine, restored the control minute volume within 2 minutes and this improvement was practically maintained for the rest of the test period.

When the antidote was given simultaneously with alphaprodine, the highest values of minute volume were obtained 15 minutes after the drugs' administration, with all three dose levels of the antagonist.

The left hand portion of Section D of Fig. 4 shows that there was a marked difference between the mean minute volumes 2 minutes after the administration of alphaprodine, when no levallorphan was given, and when the antagonist was administered.

As appears from the right hand portion of Section D of Fig. 4, the mean minute volumes, at 10 minutes after alphaprodine, were quite similar at all three dose levels of levallorphan when the antagonist was given together with or before the narcotic. However, the increase of minute volume was commensurate with the dose of levallorphan when the antagonist was administered 2 minutes after alphaprodine.

Alveolar ventilation rates were calculated, because from the viewpoint of oxygenation and especially of CO_2 removal, these measurements are of greater importance than minute volumes. As was shown, alphaprodine alone affected alveolar ventilation to a greater extent than minute volume. In contrast, the protection afforded by levallorphan against alphaprodine-induced depression of alveolar ventilation and of minute volume was of the same order of magnitude.

From these findings it appears that

levallorphan is an effective antagonist against respiratory depression caused by alphaprodine. When given as an antidote for alphaprodine-induced respiratory depression, a dose of levallorphan 1/25th that of alphaprodine produced optimal results. Although the prophylactic use of levallorphan prevented respiratory depression to a large extent, such prophylactic use of the antidote involves the question of its antagonism against other effects of alphaprodine, particularly its analgesic action. Investigations which will throw light on this problem were initiated by us.

The effectiveness of an antagonist in counteracting narcotic-induced respiratory depression may be tested in at least two ways, namely, by comparing the values of respiratory rate, minute volume and alveolar ventilation after administration of the narcotic alone and in combination with the antagonist, or by measuring the response of the respiratory center to CO_2 stimulation under the influence of the narcotic alone and combined with the antagonist. In the present study, which was undertaken primarily to determine the usefulness of levallorphan in counteracting alphaprodine-induced respiratory depression if this analgesic is administered for supplementation of N_2O-O_2-thiopental sodium anesthesia, the first method was chosen. The reason for this is that under general anesthesia there is little likelihood of a significant increase in CO_2 production. Consequently, as long as the airway is unobstructed, the patients' respiratory rates and minute volumes are kept at approximately normal levels, and as long as the exhaled CO_2 is adequately removed, either by absorption or by washing out with high gas flows, there is no danger that the respiratory center will be subjected to the drive of high CO_2 tensions. Under the experimental conditions described, levallorphan afforded a highly significant, although not complete, protection against the respiratory depression of alphaprodine. It has recently been shown by Hamilton and Cullen[7] and by Gross and Hamilton[6] that levallorphan affords similar protection against the respiratory effect of other analgesics (levo-Dromoran tartrate, morphine, meperidine) under circumstances similar to our experimental conditions.

Thomas and Tenney[12] recently reported that the antagonism of levallorphan against levo-Dromoran-induced respiratory depression can also be demonstrated when the respiratory mechanism is subjected to stress. These investigators found that in unanesthetized human volunteers, who breathed, respectively, room air, 2½% CO_2 in O_2 or 5% CO_2 in O_2, the protective effect of levallorphan became more manifest under the drive of CO_2 and was greatest when the alveolar CO_2 tension was highest.

Landmesser *et al.*[9] showed that in patients, who had received morphine or levo-Dromoran tartrate as supplement to N_2O-O_2 anesthesia, the administration of levallorphan produced a marked decrease in the arterial CO_2 content.

The data presented, together with the findings of Hamilton and Cullen[7], Gross and Hamilton[6], Landmesser *et al.*[9], and Thomas and Tenney[12], indicate that levallorphan, whether used under conditions encountered in unanesthetized or anesthetized patients, or in volunteers, whose respiratory mechanism is driven by CO_2, affords significant protection against the respiratory depressant action of narcotics.

Summary and Conclusion. A study of the effect of Nisentil HCl and of the narcotic antagonist, levo-3-hydroxy-N-allylmorphinan tartrate (levallorphan

tartrate), on respiration was carried out on 210 conscious patients, who underwent surgery under low spinal or epidural anesthesia.

In the first part of the investigation the changes of respiratory rates were measured in 120 subjects who breathed room air. They were given 1 mg./kg. of Nisentil HCl as a standard dose, except for a few debilitated subjects who received 0.75 mg./kg. In 40 patients each, Nisentil HCl was administered before, together with, or after levallorphan tartrate.

In these three groups, 20 subjects each received 0.01 and 0.02 mg./kg. of the antagonist. Thus, the ratios of Nisentil HCl to levallorphan tartrate were 100 : 1 and 50 : 1. The results were as follows:

1. Nisentil HCl caused a marked depression of the respiratory rate.

2. Levallorphan tartrate given after, together with, or before Nisentil HCl corrected or prevented the fall of respiratory rate to a large degree.

3. There was no marked difference between the effects of the two dose levels of levallorphan tartrate.

4. No consistent significant effects on pulse rate or blood pressure were observed.

In the second part of the study kymographic tracings were made of the respirations of 90 patients who breathed oxygen in a closed circuit. One mg./kg. Nisentil HCl was administered to all subjects. In 30 patients each, Nisentil HCl was administered before, together with, or after levallorphan tartrate. In these three groups, 10 subjects each received 0.01, 0.02 and 0.04 mg./kg. of the antagonist. Thus, the ratios of Nisentil HCl to levallorphan tartrate were 100 : 1, 50 : 1 and 25 : 1. Respiratory rates, tidal volumes, minute volumes and alveolar ventilation were studied. The results follow:

1. Nisentil HCl affected alveolar ventilation rate, minute volume, and respiratory rate to a greater extent than depth of respiration.

2. Levallorphan tartrate, given after, together with, or before Nisentil HCl, corrected or prevented the respiratory depression to a considerable degree. The antagonist was more effective in counteracting the decrease in depth of respiration than in rate.

3. Best effects on Nisentil HCl-induced depression of respiratory rate were obtained with the 0.04 mg./kg. dose of levallorphan tartrate. Similarly, this dose level of the antagonist, if given together with or before Nisentil HCl, afforded greater protection against depression of respiratory rate than the two lower dose levels.

4. At all three dose levels, levallorphan tartrate corrected or prevented the Nisentil HCl-induced decrease of tidal volume.

5. Best effects on Nisentil HCl-induced decrease of minute volume were obtained with the 0.04 mg./kg. dose of levallorphan tartrate. However, if given together with or before Nisentil HCl, all three dose levels of levallorphan tartrate afforded approximately the same degree of protection against depression of minute volume.

6. Although Nisentil HCl affected alveolar ventilation to a greater extent than minute volume, the degree of protection afforded by levallorphan tartrate against Nisentil HCl-induced depression of alveolar ventilation and of minute volume was of the same order of magnitude.

It is concluded that levallorphan tartrate offers considerable protection against Nisentil HCl-induced respiratory depression.

ACKNOWLEDGMENT: We are indebted to Dr. L. A. Pirk of Hoffmann-La Roche, Inc., for his unrelenting efforts in connection with this work.

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SUMMARIO IN INTERLINGUA

Effectos de Nisentil-Hydrochlorido e Tartrato de Levallorphano Super le Respiration

Esseva studiate le effectos de Nisentil-Hydrochlorido e de tartrato de levallorphano (tartrato de levo-3-hydroxy-N-allylmorphinano) super le respiration de 120 patientes exponite al atmosphera natural e de 90 patientes recipiente 100 pro cento oxygeno. Nisentil-Hydrochlorido esseva administrate intravenosemente in doses de 1 mg per kg de peso corporee ante, con, o post le administration intravenose de tartrato de levallorphano in doses de 0,01, 0,02, o 0,04 mg per kg de peso corporee.

Nisentil sol causava marcate depressiones del rapiditate respiratori, del volumines per respiration e per minuta, e del ventilation alveolar. Inter istos le depression del volumine respiratori esseva le minus marcate. Le administration de tartrato de levallorphano—in omne le mentionate doses e combinationes con Nisentil—resultava in un considerabile correction o mesmo prevention del depression respiratori. Le effecto antagonistic de levallorphano in doses de 0,04 mg per kg esseva superior al effecto del mesme droga in doses de 0,02 mg per kg. Le effecto del dos de 0,01 mg per kg esseva le minus pronunciate. Ben que Nisentil habeva un plus grande effecto super le ventilation alveolar que super le volumine per minuta, le action protective de levallorphano esseva in ambe casos del mesme grado general de magnitude.

HOSPITAL USAGE OF BLOOD

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Procedures for judging if the therapeutic use of human blood runs comparatively high or low per annum have received intermittent analytical attention since early in 1947. At that time the Advisory Board on Health Services of the American National Red Cross carefully weighed a proposal to activate civilian regional blood programs whenever the interested chapters, medical societies, hospitals, and public health authorities so requested. Guided by experience in Massachusetts[1], North Dakota, and Michigan, where chapters had sponsored programs operated by the respective state health departments, the Board speculated that blood sufficient for 5 transfusions per bed in general hospitals and one per bed in hospitals for tuberculous or mental patients would assure an adequate supply in any contemplated region. To date no one has specifically pointed evidence toward confirming or refuting the usage rates then envisioned. We, therefore, turn to the figures (Table 1) pertaining to 1,837 general hospitals and 198 other hospitals that received their entire supply from the Red Cross in the fiscal year July, 1953, through June, 1954.*

Small, general hospitals depended most numerously on the Red Cross Service, 70% being less than 100 beds each. Here and there in hospitals connected with medical schools and in others that met heavy surgical requirements the quantity per bed rose appreciably above the average (5.39 pints) but rarely reached 10 pints. A moderately strong tendency for the relatively high rates to be associated with large total bed capacity persisted despite frequent nonconforming exceptions, ascribed to unequal patient loads, differences among physicians with respect to employing blood therapy, and numerous unidentifiable causes.

Manifesting no relationship between usage rate and bed capacity, the larger and less numerously served hospitals outside the general category presented a marked contrast. Neuropsychiatric patients, affected with disabilities that usually entail treatment without blood therapy, receive transfusions only as the exigencies of surgery, blood dyscrasias, and injuries require. The same qualifications apply to tuberculous patients except that blood replacements after hemorrhaging govern their transfusions to a somewhat greater degree. Under such conditions the low average quantity per bed (.31 of a pint) warrants acceptance notwithstanding the unavoidably small and heterogeneous sample considered. Never has the observed annual rate exceeded a fraction

*In the year, 2,071 hospitals depended on Red Cross for their entire blood supply, and 1,476 for either supplementary or emergency supply, making 3,547 in all. Total use amounted to 1,522,970 pints, estimated to have been administered to approximately 761,500 patients. (Here a pint denotes a quantity 480 cc. in volume.) The completely served hospitals represented in the table are those for which bed capacities were ascertained. All data relate to the operations of 45 regional programs.

of a pint for hospitals outside the general class.

Responding to medical interest in yardsticks for measuring blood usage, we can report statistical implications from data on 1,396 general hospitals totally supplied by Red Cross in the to disclose stronger relationships than in providing objective indications that blood usage in general hospitals has progressed far toward consistency in the short period elapsing since civilian blood programs began to come into existence in the wake of World War II.

TABLE 1.—AVERAGE BLOOD USAGE IN DESIGNATED NUMBERS OF GENERAL AND OTHER HOSPITALS, BY SIZE CLASSES (BED CAPACITY); JULY, 1953, THROUGH JUNE, 1954

Size class (Beds)	*Average beds*	*Average pints*		*Hospitals*		
		Per bed	*Per hospital*	*Number*	*Percentage of total*	*Cumulative percentage*
General						
Under 25	17	4.29	73	296	16.1	16.1
25— 49	35	5.17	181	468	25.5	41.6
50— 74	58	5.55	322	335	18.2	59.8
75— 99	85	5.75	489	190	10.3	70.1
100—124	108	5.60	626	141	7.8	77.9
125—149	134	6.17	827	84	4.6	82.5
150—174	159	6.31	1,004	72	3.9	86.4
175—199	185	6.74	1,246	33	1.8	88.2
200—299	237	6.32	1,497	120	6.5	94.7
300—399	344	7.99	2,756	43	2.3	97.0
400—499	439	5.82	2,557	16	.9	97.9
500 and over	807	2.22	1,789	39	2.1	100.0
All classes	100	5.39	537	1,837	100.0	..
Other						
Under 25	13	6.18	81	17	8.6	8.6
25— 49	35	4.26	148	25	12.6	21.2
50— 74	61	3.04	184	17	8.6	29.8
75— 99	83	2.15	178	13	6.6	36.4
100—124	109	1.07	118	11	5.6	42.0
125—149	132	3.77	498	6	3.0	45.0
150—174	157	1.84	290	11	5.6	50.6
175—199	194	1.79	346	4	2.0	52.6
200—299	236	1.28	302	23	11.6	64.2
300—399	355	1.37	486	9	4.5	68.7
400—499	442	1.38	612	8	4.0	72.7
500 and over	2,307	.06	149	54	27.3	100.0
All classes	729	0.31	229	198	100.0	..

calendar year 1952. The squared variations in number of beds per hospital reflected corresponding variations in blood usage with an efficacy of 61%. Employing patient admissions as an alternative criterion, 59% resulted. Then with beds and admissions brought together as simultaneous criteria the efficacy rose to 73%. The importance of these findings lies less in their failure

Divers techniques enable the staffs at national headquarters and the area officers of the Red Cross to see how the total blood usage in the general hospitals served within a designated region compares with mean usage for hospitals having the same aggregate bed capacity. These techniques also provide tools for securing impressions concerning the demand for blood in

regions that lack well-organized collection and distribution programs. Of course the analyst who is interested in the quantity likely to be wanted for transfusions in a specific hospital faces a more precise problem. He must work with an experience table for that hospital, based on the types and frequency of cases requiring blood therapy. The medical and surgical categories adopted by Straus and Torres[2] (or some similar classification) seem to be well adapted to that purpose.

Summary. An analysis of hospital usage rates of blood for transfusion purposes has been presented.

The quantity of blood required per bed averaged 5.39 pints for general hospitals totally supplied by the American Red Cross in the period from July, 1953, through June, 1954. This quantity contrasted with only 0.31 pint per bed for hospitals outside the general category.

Blood usage in general hospitals bore a slightly stronger statistical relation to their bed capacities than to patient admissions.

There is evidence that the usage in general hospitals has made substantial progress toward consistency since World War II.

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SUMMARIO IN INTERLINGUA

Le Consumption de Sanguine per le Hospitales

Es presentate un analyse del requirimentos quantitative de sanguine pro transfusiones in le hospitales del Statos Unite.

Le datos es exprimite in pintas representante volumines de 480 cm cubic.

In hospitales general que recipeva omne lor sanguine ab le Cruce Rubie American le consumption de sanguine per lecto esseva, 5,39 pintas durante le periodo de un anno ab julio 1953 usque al fin de junio 1954. In hospitales non includite in le categoria de "hospitales general" le correspondente quantitate esseva solmente 0,31 pintas. Le consumption de sanguine del hospitales general monstrava un levemente plus forte relation statistic al numero de lectos que al numero de patientes admittite.

Le datos indica que le uso de sanguine in le hospitales general ha progredite verso valores plus uniforme depost le fin del secunde Guerra Mundial.

THE USE OF PENTAERYTHRITOL TETRANITRATE IN CHRONIC CORONARY INSUFFICIENCY

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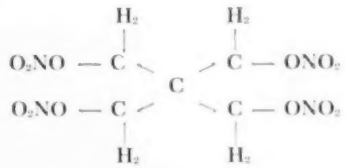
The therapy of chronic coronary insufficiency is primarily concerned with the establishment of compensation in the coronary circulation. The treatment must, of necessity, be individualized, and must consider all factors involved in the development of the disease and its resulting symptoms. In some instances the correction of underlying secondary factors (anemia, cardiac decompensation, obesity, and the like) may completely eradicate the symptoms characterized largely by the syndrome of angina pectoris or its equivalent. In others, in spite of all the physical and pharmacologic agents at our disposal, we find that many patients continue to be disabled in varying degree.

The lack of adequate treatment for this latter group prompted us to consider a drug which might be of therapeutic value. Pentaerythritol tetranitrate (Peritrate)* appeared to us as a possible answer to this problem, and it was with that purpose in mind that the present study was undertaken.

Chemically, pentaerythritol tetranitrate has the following structure:



The drug is a nitric acid ester of a tetrahydric alcohol, penta erythritol, and therefore, is found in the same group with nitroglycerine, erythritol tetranitrate, and mannitol hexanitrate. It has been found to be stable as compared with erythritol tetranitrate and nitroglycerine and is absorbed slowly in the intestines[1,8]. The physiologic effects of this drug are much less marked than with nitroglycerine and are similar to those noted with erythritol tetranitrate. Previous studies evaluating the clinical effects of pentaerythritol tetranitrate have been reported with excellent results[2,3,5-7,9].

Methods and Materials. Forty-two patients were originally studied. Twenty patients continued in the study for approximately 6 months. These patients had an average of 60.2 years, ranging from 48 to 75. Nineteen of the 20 patients were males. All patients had clinically obvious angina pectoris for

*The Peritrate was generously supplied by Dr. Walter Hoskins of the Warner-Chilcott Laboratories.

which they all had previously sought medical attention. All patients, despite varying frequency or intensity of their pain, got immediate and complete relief from nitroglycerine taken sublingually although some complained bitterly of the side-effects, especially headache. Thirteen (65%) of the patients, all males, had had proven myocardial infarction at some time prior to the study period. No patient manifested any degree of cardiac failure during the study period, although 12 were on maintenance digitalis therapy. No situations arose where the use of mercurial diuretics were necessary. One patient (5%) expired during the study period. His death was attributed to an acute myocardial infarction and the diagnosis was proven by necropsy.

The patients were studied for 935 days on pentaerythritol tetranitrate and 595 days on placebo. The duration of an individual study ranged from 30 to 182 days. The patients were required to keep an accurate daily report card, recording the severity of pain and the number of nitroglycerine tablets taken during each 24-hour period. They were seen by the same physician at the same time and place each week. The drug was supplied during each visit and was assigned to each patient by his number. The double blind technique was employed. Neither the attending physician nor patients knew when or for how many weeks drug or placebo was supplied. Dosage was begun at 2 tablets four times a day increasing to 3 tablets four times a day during a one-month period. Electrocardiograms were available on each patient prior to the study and were taken upon conclusion of the period. Five (30%) had previously taken Peritrate in the dosage of one tablet four times a day without any significant relief.

Method of Scoring and Results. The effect of pentaerythritol tetranitrate on the severity of pain was evaluated in the following manner. The patient's scorecard was divided into four categories: more pain than usual, usual pain, less pain than usual, or no pain. A numerical score of 4 was substituted for no pain; 3, for less pain; 2, for same; and one, for more pain. The patient's daily score for the entire study period on and off drug was calculated on this basis and the mean determined as illustrated in Table 1. Similarly, the same scoring procedure was carried out as to nitroglycerine requirement, the numerical value 4 substituted for no pills, 3 for one pill, 2 for two pills, 1 for three pills, and 0 for four or more pills. The daily score was calculated and the mean determined, as illustrated in Table 2. The mean difference comparing placebo and pentaerythritol tetranitrate was easily obtainable as illustrated in column 3 of Tables 1 and 2.

No patient exhibited side-effects or intolerance to the drug when taking 20 mg. (2 tablets) four times a day. When taking 30 mg. (3 tablets) four times a day, one patient noted drowsiness, one drowsiness and nausea, and two, nausea alone without drowsiness.

In 10 (or 50%) of the patients there appears to be a lag period ranging from 3 to 7 days before effect is noted when starting the drug. In several patients it appeared that the effect persisted for 24 to 48 hours after the drug was omitted.

During the period of increased dosage to three tablets four times a day, 3 patients improved with absence of pain and no nitroglycerine requirement. However, 2 of these patients could not tolerate the drug at this dosage due to side-effects. Side-effects were noted in a total of 4 patients. Reverting to 2 tablets four times a day caused complete remission of symptoms. Nineteen patients, upon completion of the study, were taking 2 tablets four times a day. One patient was taking 3 tablets four times a day without adverse effects.

As is illustrated in Table 1, 15 (75%) of the 20 patients exhibited a positive score but by subjecting the figures to statistical analysis only 5 (25%) (Patients 2, 3, 6, 18 and 20) were significantly improved. Table 2 illustrates that 16 (80%) exhibited a positive mean difference; however, only 2 (10%) (Pa-

TABLE 1.—SEVERITY OF PAIN

Patient	*Pentaerythritol Tetranitrate*	*Placebo*	*Difference*
1	2.77	2.80	— .03
2	2.22	1.18	1.04
3	3.94	2.75	1.19
4	3.58	3.34	.24
5	3.68	3.33	.35
6	2.88	1.85	1.03
7	2.52	3.48	— .96
8	1.93	2.76	— .83
9	2.46	2.10	.36
10	2.00	3.05	—1.05
11	3.43	2.86	.57
12	3.55	2.86	.69
13	3.75	3.57	.17
14	3.06	3.01	.05
15	3.24	2.92	.32
16	2.00	2.29	— .29
17	3.43	2.86	.57
18	3.71	2.14	1.57
19	2.86	2.14	.50
20	3.86	1.71	2.15

Calculated mean of daily score for the entire period of observation A higher score indicating more pain, and a lower score less pain.

TABLE 2.—NITROGLYCERINE REQUIREMENT

Patient	*Pentaerythritol Tetranitrate*	*Placebo*	*Difference*
1	3.39	3.17	.22
2	2.71	.59	2.18
3	3.93	2.38	1.55
4	3.63	3.06	.54
5	3.87	3.45	.42
6	3.20	2.72	.48
7	2.48	3.31	— .83
8	1.79	1.71	— .08
9	2.91	3.49	— .58
10	2.66	2.71	— .05
11	3.17	2.46	.71
12	3.02	3.20	— .18
13	3.68	3.50	.18
14	.28	0.00	.28
15	2.24	2.07	.17
16	.85	.35	.50
17	3.50	3.00	.50
18	3.67	3.00	.67
19	1.77	0.71	1.07
20	3.58	0.14	3.44

Calculated mean of daily score for the entire period of study. A score of 0 indicating four or more nitroglycerine tablets ingested daily, and a score of 4 indicating no tablets ingested.

tients 2 and 20) were proven to have statistically valid improvement.

Comments and Conclusions. Prolonged observation of patients with coronary artery disease who manifest the symptom complex of angina pectoris reveals that there are innumerable factors which are responsible for the production of symptoms. These are of such variable and inconstant nature, that the evaluation of any remedial agent must of necessity be interpreted broadly as it affects the character and the course of this disease. We have attempted to be most objective and as much as possible have tried to substitute figures and use statistical analysis in the interpretation of our results.

Prior to subjecting our results to analysis, we had an over-all impression that the majority of our patients were benefited considerably by pentaerythritol tetranitrate. The enthusiastic comments of the patients about the added comfort, increased relief from pain, and relative elimination in the frequency and quantitative need for nitroglycerine prompted this opinion. The apparent innocuous effect of the placebo could be inferred most of the time (even though we never knew specifically when either placebo or drug were being ingested) by the positive score they were showing on their report cards. However, only 5 (25%) patients showed improvement which was of such significance that the drug would be considered of definite and distinct value. Of the remaining patients, 11 (55%) showed positive scores, which did not, however, prove significant. The remaining 4 (20%) patients showed no effect whatsoever.

In considering the progress of these patients, we became aware that there may be a time lag of from three to seven days from the beginning of the ingestion of pentaerythritol tetranitrate until its full effects could be noted. We also observed that there might be a carry-over of the therapeutic effect for 1 or 2 days after the omission of the drug.

Summary. A clinical study of the effects of pentaerythritol tetranitrate in the dosage of 20 mg. four times a day to 30 mg. four times a day was performed utilizing the double-blind technique during a period of 6 months.

Five (25%) patients showed significant improvement to indicate a definite need for the drug. Eleven (55%) patients expressed opinions which were favorable, but when their replies were exposed to statistical analysis, they proved to be equivocal.

Dosage of 30 mg. four times a day appeared to be more effective in 3 (15%) of patients, but side-effects prevented continuation of the drug in 2 of these, side-effects were absent in all patients in the dosage of 20 mg. four times a day. A lag in therapeutic effect of from 3 to 7 days after the drug was started was seen in 50% of the patients, also a carry-over of effect after omission of from 1 to 2 days was noted.

From our study we conclude that pentaerythritol tetranitrate was of decided value in the treatment of 25% of patients with chronic coronary insufficiency. Except for the findings of Riseman[4], the results are significantly different from those previously reported. However, because of the difficulty in managing this disease adequately with the therapies now available, we feel that this drug should be considered seriously as a positive addition to the pharmacologic treatment of angina pectoris. We feel that in some patients with this illness pentaerythritol tetranitrate may mean the difference between complete, or almost complete, absence of symptoms, or a prolonged illness with much suffering.

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SUMMARIO IN INTERLINGUA

Le Uso de Tetranitrato de Pentaerythritol in Chronic Insufficientia Coronari

Esseva executate un studio clinic del effectos de tetranitrato de pentaerythritol in chronic insufficientia coronari. Le extension del studio esseva 6 menses. Le dosages usate esseva inter 20 e 30 mg quatro vices per die. Le controlos esseva "duple-cec."

Cinque patientes (25 pro cento) exhibiva meliorationes significative, indicante clarmente le indispensabilitate del droga. Dece-un patientes (55 pro cento) exprimeva opiniones favorabile, sed le analyse statistic de lor responsas los demonstrava equivoc.

Dosages de 30 mg quatro vices per die pareva plus efficace in 3 (15 pro cento) del patientes, sed in 2 de illes, effectos lateral preveniva le continuation del medication. Nulle effectos lateral esseva notate in le patientes recipiente dosages de 20 mg quatro vices per die. In 50 pro cento del patientes il occurreva un retardo del effecto therapeutic de inter 3 e 7 dies post le initiation del droga. Post interruptiones del medication pro inter 1 e 2 dies le effecto remaneva continue.

Nos conclude ab nostre studio que tetranitrato de pentaerythritol esseva clarmente de valor in le tractamento de 25 pro cento del patientes con chronic insufficientia coronari. Non considerante le constatationes de Riseman, nostre resultatos differe significativemente ab illos previemente publicate. A causa del difficultates de tractar le morbo adequatemente per medio del therapias nunc disponibile, nos opina que iste droga deberea esser considerate seriemente como addendum al serie de pharmacos recommendabile in le tractamento de angina de pectore. Nos crede que in certe patientes con iste morbo le uso o non-uso de tetranitrato de pentaerythritol corresponde possibilemente al differentia inter le complete o quasi-complete absentia de symptomas e un prolongate maladia con grande suffrentias.

THE TREATMENT OF ANGINA PECTORIS WITH A NITROGLYCERIN OINTMENT

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Angina pectoris by well established definition consists of a particular type pain due to temporary discrepancy between oxygen supply and oxygen need of the heart. Therapy of the acute attack has consisted of the use of the rapid-acting nitrites, glyceryl trinitrate (nitroglycerin) and amyl nitrite. The second aspect of treatment invokes the prevention of attacks by such general measures as avoidance of physical and emotional strain, overeating and obesity. Rapid-acting nitrites, mainly nitroglycerin in the tablet form, are given prophylactically before such physical or mental efforts prone to cause attacks. The short duration of action of these drugs limits their efficacy, and the nitrites with a longer duration of action, such as erythrol-tetranitrate and mannitol-hexanitrate, have long been a part of prophylactic therapy. These latter have undesirable side effects in many patients and are often ineffective, and in recent years several modifications have been introduced in an attempt to improve the prophylactic aspects of drug therapy. Results are difficult to evaluate, and thus far no one drug can be regarded as the solution to this problem.

This difficulty with the long-acting nitrites is exemplified in the authors' treatment of a case of coronary insufficiency following acute coronary thrombosis in March, 1953. Pain in the chest was almost constant despite the use of 15 to 20 nitroglycerin tablets a day, as well as full dosage of the longer acting nitrites. Opiates in large dosage had to be employed to give the patient any relief at all. A cardiac consultant agreed that the pain was that of coronary insufficiency and that some further method of securing vasodilatation of the coronaries was essential. Because of the ineffectiveness of the conventional measures, it was suggested that a 2% nitroglycerin ointment be applied to the skin[2]. This ointment had been reported as being of value in disease of the peripheral circulation by Fox[1], Kleckner[3], and Lund[4], and it was hoped that the slowed absorption through the skin might result in the superior vasodilating effect of nitroglycerin being prolonged in relation to the coronary circulation. The results in this case were dramatic and convincing enough to have impelled us to employ it in the management of subsequent problem cases of coronary insufficiency with angina pectoris since this time.

Clinical Material and Method. Seventeen patients have been observed for a sufficient time to evaluate the effect of this mode of usage in conjunction with the other usual

measures, including nitroglycerin tablets sublingually as necessitated by anginal pain. Sixteen of these patients were seen in private practice and one in a general medical clinic. The diagnosis had been proven by acceptable standards in all cases. In each instance the clinical results were unsatisfactory at the time the ointment was started.

Controls are provided by the record of the number of nitroglycerin tablets required before treatment was started and similar tabulation afterwards. The authors have not felt it justifiable to substitute an inert ointment in these patients as a placebo, because of the possible danger of inducing myocardial infarction in patients with such severe angina.

Originally the ointment was prepared locally, but more recently we have employed a commercial product, Nitrol*, which is supplied in a one-ounce tube. The ointment is applied with a wooden applicator to the chest wall over an area 5 to 8 inches in diameter. The initial dosage is one-half inch of the ointment applied 3 or 4 times daily. This may be varied by increasing the length of the ribbon of ointment to three-fourths of an inch or more, or decreasing as indicated. The actual amount of nitroglycerin in such dosage has been calculated as 15 mg. in each inch of the ointment. Frequency and time of application is governed by the effects on the pain pattern. Headache is a frequent side effect at the start of treatment and has at times made it necessary to decrease the amount used in a single dose. Usually tolerance to side effects develops rapidly and reduction of dosage is only temporary. The chief indication for reduction of dosage has been improvement as gauged by a decrease in the number of attacks of anginal pain. The ointment might be applied anywhere on the body, but the chest area affords some psychological benefit as well as convenience. Methemoglobinemia has not been observed in any of these patients.

Results. It is the authors' impression that there has been definite benefit from this therapy, when used together with all other measures for the treatment of angina pectoris. The regimen of rest, the use of long-acting nitrites, the use of sedatives, and the taking of nitroglycerin sublingually were all continued as before. We have classified the patients in three groups: (1) greatly helped, (2) moderately helped, (3) not helped. This classification is based on the subjective effects reported by the patient as well as the reduction in the number of sublingual nitroglycerin tablets required.

These results may be influenced by a number of factors other than coronary insufficiency. Some patients had only arteriosclerotic heart disease while others had complicating diabetes, gallbladder disease, and other pathologic processes which might interfere with response. The mental attitude alters effects, as failure to carry out the supplemental regimen or carelessness in applying the ointment have adversely affected some cases. It has been pointed out that exposure to air or simple aging will alter the pharmacological property of the ointment as is true with nitroglycerin tablets. This has been noted by several of the patients treated, and the authors are now advocating the prescribing in one-ounce tubes to avoid this. One of the patients (Case 13) suffered a second myocardial infarction while using the ointment. Shortly before the attack he noted a change in effect of the ointment and increased the amount used from one-half to three-quarters of an inch. We believe that this would indicate caution in discontinuing the ointment suddenly, as coronary insufficiency might be suddenly increased and result in myocardial infarction.

The following cases are illustrative of those considered to have shown good results.

Case Reports. CASE 1. P.W.T., a white male aged 70, was a diabetic of long standing, taking 20 to 25 units of Protamine zinc insulin for a number of years. In 1942 he suffered an acute myocardial infarction. Following this, he tended to exhibit a mild elevation of blood pressure but otherwise was symptom free until 1953, at which time a

*Nitrol Ointment: Kremers-Urban Company, Milwaukee 1, Wis.

TABLE 1.—CLINICAL DATA AND RESULTS OF TREATMENT, INCLUDING NITROGLYCERIN BY INUNCTION IN 17 PATIENTS WITH ANGINA PECTORIS

Name	*Sex*	*Age*	*Living or Dead*	*Occurrence of Myocardial Infarction*	*Onset of Angina*	*Previous use of long-acting Nitrites*	*EKG*	*When Ointment Started*	*No. of Tablets of Nitroglycerin*		*Results*
									Before Use of Ointment	*After Use of Ointment*	
1. P.W.T.	M	70	L	1942 1953	1952	No	Abnormal	5/ 1/53	1–7/d	0–1/d	Good
2. J.E.C.	M	69	L	1945	1945	Yes	Abnormal	5/ 6/53	0–5/d	0–4/d	Good
3. M.L.R.	M	77	L	No	1952	Yes	Abnormal	5/ 9/53	8–10/d	0–2/d	Good
4. F.R.	M	63	D	1946 1952	1946	Yes	Abnormal	5/12/53	2–6/d	2–6/d	Poor
5. M.B.	F	62	D	No	1944	Yes	Abnormal	8/12/53	2–6/d	2–6/d	Poor
6. H.B.	M	62	L	No	1951	No	Normal	5/ 7/54	2/d	1/mo.	Good
7. S.S.	M	71	L	No	1948	Yes	Normal	6/ 8/54	4–5/d	4–5/d	Poor
8. A.L.B.	F	74	L	No	1952	Yes	Normal	6/15/54	6–8/d	2–4/d	Good
9. A.K.A.	M	42	L	1954	1954	Yes	Abnormal	8/14/54	3/d	2–3/d	Good
10. S.D.K.	M	42	L	1946	1946	No	Abnormal	8/18/54	1–2/d	2–3/2 mo.	Good
11. R.T.	M	45	L	1954	1954	No	Abnormal	8/27/54	0	0–2/d	Fair
12. B.C.	F	64	L	1953	1954	No	Abnormal	9/ 7/54	4–5/d	0–4/d	Fair
13. A.G.*	M	44	L	1950 1954	1954	No	Abnormal	9/16/54	3–4/d	1/d	Good
14. R.L.P.	M	64	D	No	1954	Yes	Abnormal	10/ 4/54	6–10/d	4–8/d	Poor
15. S.C.	F	60	L	1951	1951	Yes	Abnormal	10/18/54	0–10/d	0–4/d	Good
16. S.L.I.	M	74	L	1946	1946	Yes	Abnormal	10/19/54	25–35/d	10–12/d	Fair
17. J.T.G.	M	62	L	1953	1954	No	Abnormal	12/ 8/54	2–3/d	0–3/d	Fair

* The myocardial infarction in 1954 occurred while using nitroglycerin ointment.

second acute myocardial infarction occurred. As noted above, this patient was started on nitroglycerin ointment 2 weeks following this second acute infarction, at which time he was suffering almost constant substernal pain requiring opiates for relief. There was immediate improvement in the severity and frequency of the chest pain during hospitalization. Upon discharge he was using the ointment three times daily, but this has been reduced to one or two applications a day, an application at bedtime preventing any pain during the night. He now requires only 2 or 3 nitroglycerin tablets sublingually a week, and insofar as the coronary disease is concerned he is doing extremely well. There has been no recurrence of hypertension, despite the development of esophagitis with stricture of the esophagus requiring gastrostomy.

CASE 3. M.L.R., a white male, age 77, was first seen in March, 1953, with a history of anginal pain of one year's duration. Nitroglycerin relieved the attacks. Blood pressure was 152/60. Physical examination revealed arteriolosclerosis of the retinal vessels as well as peripheral arteriosclerosis. The electrocardiogram showed S–T segment depression in leads 1 and 2, with T wave changes in leads 1 and 2, and the unipolar chest leads. For two months he averaged 8 to 10 nitroglycerin tablets per day and was particularly bothered by pain upon reclining at night. This pain was of such a degree as to interfere with sleep. The ointment was started in May, 1953, and at first was used only at bedtime. This resulted in complete cessation of the attacks at night and a general feeling of improvement. Several months later daytime attacks became more frequent and the use of the ointment was increased to three times daily. There was almost immediate decrease in the frequency of anginal attacks, and he now requires a nitroglycerin tablet only every third or fourth day. He has reduced the use of the nitroglycerin ointment to one or two times a day and at bedtime.

CASE 10. S.D.K., a white male, aged 42, was first seen in June, 1946, with an extremely severe acute myocardial infarction. He responded well to therapy, with a return of the electrocardiogram to normal. However, in late 1946 he developed substernal discomfort on exertion or with emotional upset, which responded to nitroglycerin sublingually. These attacks for a time were not severe or frequent enough to be incapacitating. They began to become much more frequent in August, 1954, and he then averaged taking one to 4 nitroglycerin tablets daily. At this time he was placed on nitroglycerin ointment three times daily. The ointment has gradually been reduced, and he now uses it only once daily at bedtime. For the past 2 months he has required 2 nitroglycerin tablets although he has maintained his usual activity and occupation.

The following cases illustrate poor results.

CASE 4. F.J.R., white male, aged 63, had an acute coronary occlusion in 1946 and a second episode in 1952. Following the first attack in 1946, he developed typical angina pectoris and later chronic congestive failure. Throughout this time, however, he continued his usual regimen with slight modifications as to diet and rest. He was taking from 2 to 6 nitroglycerin tablets a day in May, 1953, as well as a long-acting nitrite. The ointment was prescribed, but he did not follow directions as to frequency of application and was uncooperative because of the unpleasantness related to the ointment remaining on the skin. He discontinued its use 4 months after it was started as he felt that there was no improvement from its use. He died suddenly from a third acute myocardial infarction several months after discontinuing its use.

CASE 5. W.M.B., white female, aged 62, was diagnosed angina pectoris and cholecystitis with cholelithiasis in 1946. Surgery was declined at that time. The pain did not become frequent or severe until June, 1953, at which time she was having 6 to 9 nitroglycerin tablets per day. She was then hospitalized during which time she never required more than 3 nitroglycerin tablets daily. During hospitalization she was started on the ointment preparation three times daily. After leaving the hospital the frequency of the attacks increased again and exertion as slight as stooping over to reach a cabinet or climbing 3 to 4 steps would precipitate attacks. There was no apparent benefit from the ointment during its use for 2 weeks. She became completely incapacitated and cholecystectomy was resorted to in hope that the anginal pain with resultant physical and mental distress might be relieved. She expired of acute pulmonary edema four hours postoperatively.

Summary and Conclusions. A series of seventeen patients with angina pectoris have been treated with a 2% nitroglycerin ointment in addition to usual measures. Nine of these patients showed a decrease in the number of at-

tacks; 4 patients had no apparent decrease in the number of attacks but had a general feeling of well-being; 4 patients showed no response. This measure is suggested as an adjuvant in the management of coronary insufficiency. The principle of slower absorption with resulting prolonged action of an effective coronary vasodilator would seem to deserve further trial in this condition. The observation that sudden withdrawal of this ointment has produced more severe coronary insufficiency indicates to the authors that there is a marked vasodilating effect and that when it is employed, caution must be observed in sudden withdrawal because of the danger in inducing acute coronary insufficiency or an actual myocardial infarction.

ACKNOWLEDGMENT. The authors would like to gratefully acknowledge the invaluable cooperation and advice in the study and preparation of this paper given by Dr. Tinsley R. Harrison, Professor of Medicine, Medical College of Alabama, Birmingham, Alabama.

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SUMMARIO E CONCLUCIONES IN INTERLINGUA

Le Tractamento de Angina de Pectore per Medio de un Unguento a Nitroglycerina

Un serie de 17 patientes con angina de pectore esseva tractate con un unguento a 2 pro cento de nitroglycerina addite al mesuras therapeutic usual. Le resultato esseva que 9 patientes monstrava un reduction del numero de attaccos; 4 patientes non habeva un reduction del numero de attaccos sed reportava un sensation general de ben-esser; 4 patientes monstrava nulle reaction del toto.

Le uso del unguento es recommendate como adjuvante in le tractamento de insufficientia coronari. Le principio del reducite rapiditate absorptive con resultante prolongation del action de un efficace vasodilatator coronari pare meritar experimentos additional in casos del condition mentionate. In le opinion del autores, le facto que le subite cessation del uso del unguento resultava in un plus sever insufficientia coronari indica que le unguento ha un marcate effecto vasodilatatori de maniera que, quando illo es usate, su elimination debe esser effectuate cautissimemente a causa del risco de un resultante acute insufficientia coronari o mesmo de un ver infarcimento myocardiac.

COBALTOUS CHLORIDE IN THE TREATMENT OF CERTAIN REFRACTORY ANEMIAS

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and

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The hematopoietic stimulating effect of cobalt, although recognized for some time, aroused relatively little interest until a number of recent clinical observations, abroad and in this country, reported encouraging results in the treatment of anemias secondary to protracted infections and chronic diseases. Successful therapeutic trials with cobaltous chloride were reported by Berk *et al.*[1] in anemias secondary to arthritis, tuberculosis, carcinoma of the stomach and in familial microcytic anemias; Robinson, James and Kark[11] used cobalt in 9 patients with anemia due to chronic suppurative disease and found resulting increase in erythrocyte counts, hemoglobin values and hematocrit levels. Gardner[5] treated 17 patients with chronic renal disease, anemia and severe nitrogen retention; most patients showed a significant erythropoiesis within one month and previously required blood transfusions became unnecessary. Muratore[8] treated 14 cases of Mediterranean anemia with oral or intravenous cobalt and observed a rapid reticulocyte response and marked erythrocyte increase. Weissbecker[17] reported a total of 126 cases with remissions in hemolytic anemias and notable improvement in anemias due to chronic infections, leukemias and carcinoma. Wolf and Levy[19] treated 4 patients with sickle cell anemia and elicited a "dramatic" symptomatic improvement in 3 cases and "good" hematologic response in all 4 subjects. Stohlman and Rath[13], on the other hand, report only one temporary remission and 9 failures in 10 patients with sickle cell anemia given cobaltous chloride.

The object of the present study was to determine the erythropoietic activity of cobalt in several disease states, with chronic anemia as a prominent feature, necessitating in many instances repeated transfusions. The main interest was to observe gross effects of cobalt upon circulating red blood cells and hemoglobin in patients in whom other therapeutic measures had previously failed.

Materials and Methods. The subjects of this study listed in the table were regular admissions to the Medical Service of the Veterans Administration Hospital, Martinsburg, W. Va. All were males with ages ranging from 25 to 64 years. Among the patients studied were 4 with chronic glomerulonephritis, 1 with chronic pyelonephritis concurrent with hepatic cirrhosis and exfoliative dermatitis, 2 with rheumatoid arthritis and 1 with Hodgkin's disease. Hematologic studies included erythrocyte and leukocyte counts, differential white cell counts, hemoglobin and hematocrit determinations. Sternal marrow aspirations were done in several patients for diagnostic purposes.

Cobaltous chloride was prepared by the hospital pharmacy in 0.5% aqueous solution and given as such 4 times a day, after meals

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TABLE 1.—PATIENTS AND RESPONSE TO COBALT THERAPY

Patient	*Age*	*Diagnosis*	*Main Therapy pre Cobalt*	*Cobaltous Chloride Dosage and Duration*	*Hematocrit pre Cobalt*	*Hematocrit post Cobalt*	*Results*
1. P.W.	31	Glomerulonephritis, chronic.	Transfusions	0.08 gm. q.d. 66 days	23.5	31.5	Rise in hematocrit without transfusions. Died 66 days after onset of cobalt therapy from hemorrhage.
2. J.M.	62	Glomerulonephritis, chronic; nephrotic stage.	ACTH, nitrogen mustard, transfusions.	0.10–0.20 gm. q.d. 240 days	29.0	42.0	Anemia corrected. Patient died 10½ months after start of cobalt therapy with renal failure.
3. O.W.	25	Glomerulonephritis, chronic. Rheumatic carditis.	Transfusions	0.125 gm. q.d. 135 days	25.0	40.0	Anemia corrected. Died 135 days after onset of cobalt therapy in renal and cardiac failure.
4. C.G.	32	Glomerulonephritis, chronic.	Symptomatic	0.125 gm. q.d. 70 days	28.0	40.0	Disappeared from study after 70 days. Good symptomatic response.
5. L.Y.	64	Pyelonephritis, chronic. Hepatic cirrhosis. Exfoliative dermatitis.	Antibiotics	0.125–0.18 gm. q.d. 105 days	33.0	50.0	Good symptomatic response.
6. J.C.	42	Rheumatoid arthritis.	Symptomatic	0.08 gm. q.d. 108 days	35.0	42.0	Good symptomatic response.
7. J.H.	60	Rheumatoid arthritis. Prostatitis.	Symptomatic Antibiotics	0.08–0.15 gm. q.d. 135 days	34.0	44.0	Patient asymptomatic.
8. M.L.	32	Hodgkin's disease.	Transfusions. Nitrogen mustard.	0.12–0.24 gm. q.d. 225 days	25.0	39.5	Anemia improved. Patient died 13 months after onset of cobalt therapy at home of "pneumonia."

and at bedtime. No attempt was made to mask the taste of the preparation, as the solution was generally well tolerated. Initial daily dose was 0.08 gm. with gradual increase in some cases to a maximum of 0.24 gm.; maintenance doses ranged from 0.12 to 0.16 gm. daily. Periods of medication varied from 45 to 240 days, with occasional short lapses in treatment schedules. Medications prescribed previously were continued during cobalt therapy except for omission of blood transfusions. Clinical studies and observations were carried out by the authors.

nitrogen retention. All, excepting Patient 4, had been hospitalized several times previously and had received blood transfusions. The average hematocrit level presented by these 4 patients, before treatment with cobaltous chloride, was 26.4; this rose to an average value of 38.4 after cobalt administration. Increases in erythrocyte and hemoglobin values were observed

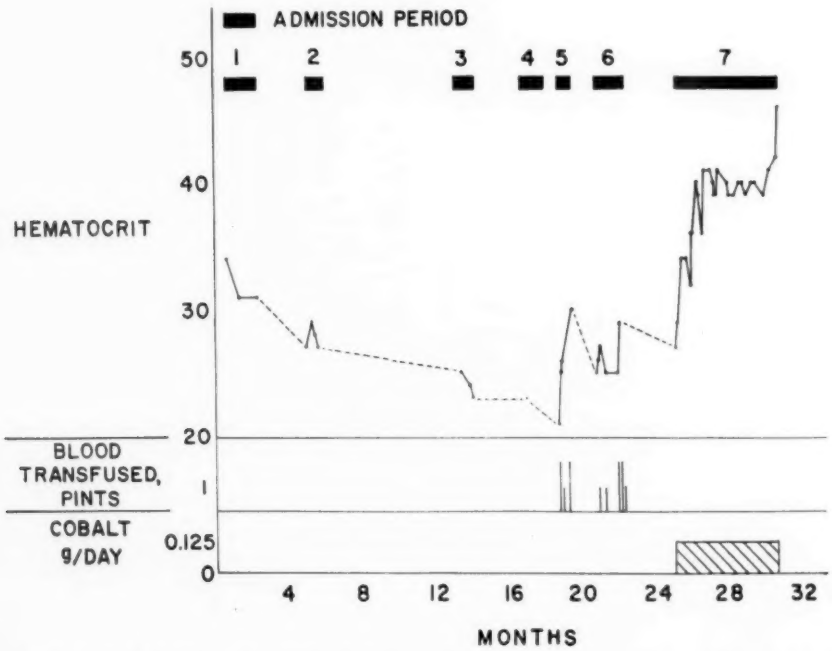

Fig. 1.—Patient 3 O.W.: Hospitalized 7 times in 32 months for chronic glomerulonephritis with nitrogen retention and anemia, and rheumatic valvular disease. Average hematocrit value 25% before cobalt therapy and frequent transfusions needed. With cobalt administration, hematocrit rose to an average of 45%. Death due to renal and myocardial failure.

Results. The average hematocrit value for the entire group was 29.1 cc. of blood prior to cobalt therapy. Folcf blood prior to cobalt therapy. Following maximum response, the average hematocrit increased to 41.3%. Pertinent data are summarized in Table 1.

The 4 patients with glomerulonephritis had advanced renal failure with

within 10 to 20 days; maximum values were reached in 30 to 40 days with apparent stabilization. Objective and subjective clinical improvement of anemia was noted and transfusions were no longer necessary. The hematologic response, however, had no apparent favorable effect upon the underlying disease and 3 of the patients died 2 to

10¼ months after start of cobalt therapy. One (Patient 3) died of azotemia and cardiac decompensation, one (Patient 2) of acute left ventricular failure, and one (Patient 1) died at home from an exsanguinating hemorrhage, details of which could not be ascertained. One case (Patient 4) was lost from observation 70 days after therapy was begun. The course of Patient 3 is illustrated in Fig. 1.

in this case was marked, and he was discharged asymptomatic.

Both patients with rheumatoid arthritis initially received symptomatic therapy only, and no appreciable change in the hematologic picture was observed. With administration of cobalt, however, the hematocrit value rose from 35 to 42 in Patient 6, and from 34 to 44 in Patient 7. Patient 6 showed an increase in erythrocytes

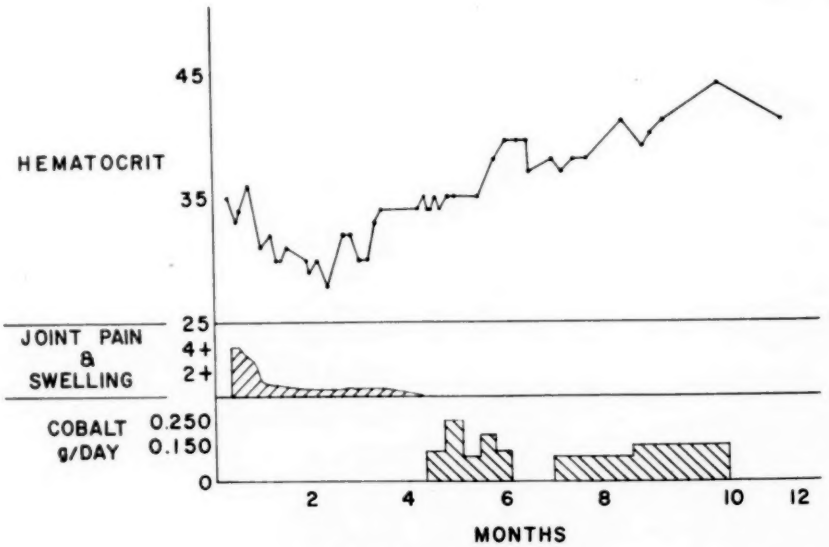

Fig. 2.—Patient 7, J.H.: Rheumatoid arthritis, chronic. Average hematocrit with symptomatic treatment stabilized at 34%. With cobalt therapy, hematocrit rose to 44%.

Patient 5, with chronic pyelonephritis, presented a severe protracted exfoliative dermatitis since 1947, following antiluetic therapy with arsphenamine. He also had a history of ethanolic excesses and of hospitalization in 1951, 1952 and 1953 because of pneumonia and cervical osteomyelitis. Prior to cobalt therapy, he presented a hematocrit value of 33; this rose to 50 following administration of the drug, dosage of which averaged 0.15 gm. a day. Rise in erythrocyte and hemoglobin values began 20 days after initiation of therapy; peak levels were reached within 45 days. The clinical improvement within 20 days, with peak values observed after 3 months of therapy. In Patient 7, the therapeutic effect was notably slower; the red cell increase began only after 40 days of cobalt therapy with maximum response reached in 5½ months. Both patients were discharged with marked general and symptomatic improvement. The clinical course in Case 7 is shown by Fig. 2.

The patient with Hodgkin's disease, in whom the diagnosis was established by histological studies of biopsied lymph nodes, entered the hospital because of severe anemia. Upon admission, the hematocrit value was 14 and

many whole blood transfusions were given with resultant rise of the hematocrit to 35. Later, a course of nitrogen mustard was administered, following which the hematocrit declined to a value of 24 within 6 weeks, despite several additional blood transfusions. Cobaltous chloride, 0.12 gm. a day, was started with a subsequent increase in erythrocyte count and hemoglobin; within 6 weeks, an hematocrit of 39 was observed. The patient was then allowed to return to work, but after 5 months of irregular cobalt intake the hematocrit level decreased to 26. Cobalt dosage was then raised to 0.24 gm. a day, which was well tolerated, and within one month the hematocrit increased to 32. During the entire period of cobalt administration the patient remained asymptomatic, requiring no other therapy. He was recently lost to follow-up study.

Discussion. Cobalt resembles iron in its physical characteristics and chemical reactions. The cobaltous salts are readily soluble and rapidly taken up by the body tissues; notable amounts of cobalt are concentrated in the liver, pancreas and spleen for short periods of time[16]. Their excretion is effected by the kidneys and the gastrointestinal tract. Trials with synthetic diets have shown that cobalt is an essential mineral needed in trace amounts for animal growth and erythropoiesis. A characteristic anemia has been described in ruminants grazing in cobalt-poor areas[4].

When administered in adequate doses, cobalt produces a true polycythemia in experimental animals and in normal humans[1,14,17]. The circulating red cell mass is increased and the erythrocytes are normochromic and normocytic[10]. Marked hyperplasia of erythrocytic marrow elements has been observed in animals in whom polycythemia was produced with cobalt. Extramedullary erythropoietic centers have been found in the liver, spleen, kidneys and suprarenal glands of cobalt-treated ducks, rats and guinea pigs[3,17].

There is no evidence that hemoglobin of animals and humans treated with cobalt is in any respect different from that of control subjects[9]. Survival studies with erythrocytes obtained from cobalt-treated patients demonstrated that these cells possessed a normal life span and acted as normal oxygen carriers[1]. A recent study[12] demonstrated that cobalt, *in vitro*, stimulates formation of methemoglobin, when incubated with normal, intact, red blood cells. There is no report of a similar action of cobalt *in vivo*.

The mode of action of cobalt is not clearly understood. Interference with transport of oxygen to erythroid bone marrow elements has been suggested[1,9,16], by combination with enzymes active in cellular respiration, or formation of oxygen-binding complexes with certain amino-acids, such as cysteine and histidine[2,9]. Warren and associates[15] suggest that cobalt might act in the liver with formation of "metabolic precursors requisite for cell production." Others[6,18] postulate that cobalt exerts its stimulating effect through mobilization and increased utilization of stored iron.

Cobalt salts do not appear unduly toxic to the animal organism. Parenteral administration of large doses has produced in humans, nausea, vomiting, and a temporary fall in blood pressure; in animals, tremors and convulsions have been observed[17]. Gardner[5] reported tinnitus and nerve deafness in 4 patients treated with cobaltous chloride; complete recovery followed when medication was discontinued. A recent report[7] describes 5 cases of thyroid hyperplasia with hypothyroidism, resulting apparently from prolonged cobalt therapy in Negro children with sicklemia. All evidence of thyroid deficiency disappeared in these cases

within several weeks after withdrawal of the drug. Other, mostly minor side effects, such as anorexia, nausea and occasional vomiting, have been reported[1,2,5] in patients given oral doses of cobalt. In the authors' experience, mild gastric distress and nausea were observed in a few instances; by progressively increasing dosage levels and administering the medication with meals or immediately after ingestion of food, these difficulties were rapidly alleviated. The low concentration of the cobalt solution used here may possibly explain the relative absence of adverse manifestations. The clinical results were encouraging, as evidenced by the hematologic response. The erythropoietic activity appeared to be directly proportional to the dose of cobalt: 0.08 gm. of $CoCl_2$ a day elicited a slower response than a dose of 0.24 gm. In our hands, cobalt appeared to be a useful and valuable drug, well tolerated and devoid of undue toxicity. Although the underlying pathologic state was not affected by the drug, all the patients showed tangible improvement, concomitant with that of hematological values.

Summary and Conclusions. Cobaltous chloride was administered orally to 8 patients with chronic, normocytic, normochromic anemia. Of these, 4 had chronic glomerulonephritis, 1 chronic pyelonephritis, 2 chronic rheumatoid arthritis and 1 Hodgkin's disease. In all patients an increase in hematocrit, erythrocyte count and hemoglobin was observed.

Daily administration of 0.12 to 0.16 gm. of cobaltous chloride, in divided doses, was found to be adequate for a satisfactory response and maintenance of attained hematologic levels.

Only minimal side-effects were observed, in the form of mild abdominal distress and slight nausea, both easily controlled by gradually increasing the dosage.

Cobalt appears to be a valuable drug in the treatment of anemias secondary to chronic diseases.

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(Interlingua on page 298)

PATHOGENESIS OF PULMONARY INFECTIONS IN PATIENTS WITH POLIOMYELITIS

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In previous publications by our group, the incidence of bacteria in the upper respiratory tract, trachea, and lungs of patients on whom a tracheotomy had been performed during the course of poliomyelitis[9] and the effects of several antibiotics on the appearance and disappearance of different species[10] of microörganisms have been reported. The facts that the organisms which were recovered at necropsy from pulmonary tissue and hilar lymph nodes, and in addition, those recovered from the blood in occasional patients with septicemia were the same as those found in the culture of the tracheal aspirate, suggested that these organisms were capable of causing significant pulmonary infection. In this paper we shall attempt to evaluate the significance of the various microörganisms in the pathogenesis of bronchopneumonia in the same patients.

Method. Specimens of pulmonary tissue were available for study from each of 27 patients with acute poliomyelitis who died. All of the patients had had difficulty with breathing and had required a tracheotomy and artificial respiration.

In each patient several blocks of tissue were obtained from each lobe of the lungs. Generally, one specimen was obtained from each of three locations: one near the periphery, one in mid zone and one near the hilum. Bacteriologic cultures were made immediately in most cases. When this was not possible, the tissues were rapidly frozen at 20° C. and stored until cultures could be made, which was always within the next 12 hours. The method of culture and media used have been given in detail elsewhere[9].

After the cultures had been obtained from each of the blocks of tissue, fixation for histologic study was completed. Multiple preparations were made from each block of tissue in order to get a representative interpretation. All histologic studies were made and the description recorded by the pathologist before the pathologic findings were correlated with the results of the cultures. The following criteria were used for pathological findings. A bacterial bronchitis was considered to be present when there was polymorphonuclear cell infiltration into and around the bronchial wall, usually with pus cells and desquamated epithelium in the lumen. Bacterial pulmonary infection was recorded as present when there were foci of bronchopneumonia characterized by polymorphonuclear cellular exudate in the alveolar spaces or

when there was infiltration of pus cells, diffusely or in clusters, into atelectatic and congested areas. Atelectasis without potential infection was recorded when there was no such accumulation of polymorphonuclear leukocytes. Other changes occasionally observed included congestion, edema, parabronchial lymphocytic infiltrations, lymphocytic infiltration of the alveolar septa and hyalin membrane formation in the alveolar space. The details and significance of these latter features are being studied in more detail. In this paper a correlation has been made between the bacteriologic findings and purulent bronchitis, and pulmonary infection (bronchopneumonia and infected atelectasis) as described above.

TABLE 1.—CORRELATION BY PATIENTS OF PATHOLOGIC FINDINGS CONSISTENT WITH BACTERIAL PULMONARY INFECTION WITH THE RESULTS OF CULTURES

		Pathologic Changes Suggesting Bacterial Infection							
	Total Patients Examined	*None*		*Minimal**		*Moderate***		*Marked****	
Results of Cultures		*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No growth									
Patients who received no antibiotic	4	3		1		–		–	
Patients who received antibiotics	4	3		1		–		–	
Total	8	6	75.0	2	25.0	0	0	0	0
Single organism obtained on culture									
M. pyogenes var. *aureus*	3	–		1		1		1	
Streptococcus viridans	1	–		1		–		–	
Aerobacter aerogenes	1	–		–		1		–	
E. coli	1	–		–		1		–	
Proteus species	1	–		1		–		–	
Total with single organism	7	0	0	3	42.9	3	42.9	1	14.3
Mixed Infection	8	–		1	12.5	5	62.5	2	25.0
Excluding *M. pyogenes* and/or *E. coli* and/or *Aerobacter aero.*									
Including *M. pyogenes* and/or *Aerobacter aero.* and/or *E. coli*	6	–		2	33.3	1	16.7	3	50.0
Total mixed infection	14	0	0	3	21.4	6	42.8	5	35.8

* Present in small patches in one or more lobes: no large areas in any lobe.
** Present in many patches in one or more lobes: at least one lobe had a few large areas.
*** Present in many confluent areas: at least one lobe markedly involved.

indicating more marked involvement was given. As can be seen, only 8 among 27 patients had completely negative cultures and 6 of these had no histologic evidence suggestive of bacterial infection. In the other 2 patients there were minor pathologic changes. Treatment with antibiotics did not appear to influence the frequency of positive cultures.

In 7 patients only one species of bacteria was isolated from the several areas cultured. In all there was some microscopic evidence of infection and in 4 the process was more extensive than any of those in the patients whose cultures were sterile. Three organisms were cultured from these 4 patients: *Micrococcus pyogenes* var. ***aureus***, *Aerobacter aerogenes*, and *Escheria coli*, once each.

Results. In Table 1 the extent of pathologic changes indicative of bacterial infection in each patient has been tabulated in relation to the bacteria isolated from all of the specimens obtained from each patient. In the compilation of this table the extent of the pathologic changes in all lobes has been considered; the final grade usually represents that of the most involved lobe except when several lobes were equally involved, in which case a rating

For this reason, when the mixed infections were tabulated, those from which one of these organisms had been obtained were separated from those

which did not contain these organisms. Fourteen patients had mixed infections consisting of various combinations of enterococci, Pseudomonas, Proteus species, *Micrococcus pyogenes* var. *aureus*, *Aerobacter aerogenes* and *Escherichia coli*. Two species were isolated from 9 of the patients, 3 from 4 patients and 5 from one patient. There was no significant difference in the pathologic changes in patients from whom one of the 3 species which caused moderate or severe pathologic changes by themselves were cultured and those from whom other microorganisms were cultured.

Since the difference in degree of pathologic change and bacteria isolated varied considerably among the different lobes in the same patient, the results were somewhat obscured by the tabulation of results for each patient.

In Table 2 the pathologic changes observed in each lobe have been compared with the microörganisms cultured from the same tissue. As can be seen, 79% of the lobes which were sterile were free of pathologic changes suggestive of bacterial infection and in the remaining 21% the changes were minimal. In 6 among 20 lobes from which a single strain of bacteria had been isolated there was more extensive infection than was found in any of the lobes with negative cultures. Organisms isolated in pure cultures from these 6 were *Micrococcus pyogenes* var. *aureus*, *Aerobacter aerogenes* and *Escherichia coli*; each of which was found in two lobes. Several species of bacteria were cultured from 31 lobes. In only one of these lobes were more than two species concurrently present. Among these lobes with mixed flora there was a significant increase in the per cent in which moderate or marked pathologic change of infection was demonstrated when com-

TABLE 2.—CORRELATION BY LOBES OF PATHOLOGIC FINDINGS CONSISTENT WITH BACTERIAL PULMONARY INFECTION WITH THE RESULTS OF CULTURES

		Pathologic Changes Suggesting Bacterial Infection							
	Total Lobes	*None*		*Minimal**		*Moderate***		*Marked****	
Results of Culture	*Examined*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No growth									
Patients who received no antibiotic	23	18	78.4	5	21.6	–	–	–	–
Patients who received antibiotics	20	16	80.0	4	20.0	–	–	–	–
Total	43	34	79.1	9	20.9	0	0	0	0
Single organism obtained on culture									
M. pyogenes var. *aureus*	7	2		3		1		1	
Streptococcus viridans	2	1		1		–		–	
Enterococcus	1	1		–		–		–	
Aerobacter aerogenes	3	–		1		2		–	
E. coli	3	1		–		2			
Proteus	4	2		2		–		–	
Total with single organism	20	7	35.0	7	35.0	5	25.0	1	5.0
Mixed Infection	18	0	0	6	33.3	9	50.0	3	16.7
Excluding *M. pyogenes* and/or *E. coli* and/or *Aerobacter aero.*									
Including *M. pyogenes* and/or *E. coli* and/or *Aerobacter aero.*	13	3	23.1	2	15.4	5	38.4	3	23.1
Total mixed infection	31	3	9.7	8	25.8	14	45.2	6	19.3

* Present in small patches and isolated areas.
** Present in many patches and foci but little confluence.
*** Present in many confluent areas.

pared with the uninfected lobes, or those from which a single strain was obtained. In only 9.7% of the lobes from which several organisms were obtained were histologic changes of a bacterial infection not seen.

As illustrated in Table 3, the microscopic pattern generally associated with a purulent bronchitis was more frequently observed in the bronchi from the sections from which positive cultures were also obtained. In the bronchi also, staphylococci, *E. coli* and mixed infections were associated with most marked evidence of infection. The severity of bronchitis generally paralleled the evidences of pulmonary infection which were present; but in one instance severe bronchitis was present without true pneumonia.

TABLE 3.—CORRELATION BY LOBES OF PATHOLOGIC FINDINGS SUGGESTIVE OF BACTERIAL BRONCHIAL INFECTION WITH THE RESULTS OF CULTURES

		Pathologic Changes Suggesting Bacterial Bronchitis							
	Total Lobes Examined	*None*		*Minimal**		*Moderate***		*Marked****	
Results of Cultures		*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No growth									
Patients who received no antibiotic	23	18	78.2	5	21.8	–	–	–	–
Patients who received antibiotics	20	12	60.0	8	40.0	–	–	–	–
Total: no growth	43	30	69.8	13	30.2	0	0	0	0
Single organism obtained on culture									
M. pyogenes var. *aureus*	7	3		1		2		1	
Streptococcus viridans	2	1		1		–		–	
Enterococcus	1	–		1		–		–	
Aerobacter aerogenes	3	–		3		–		–	
E. coli	3	–		2		–		1	
Proteus species	4	2		1		1		–	
Total with single organism	20	6	30.0	9	45.0	3	15.0	2	10.0
Mixed Infection	18	4	22.0	7	38.8	4	22.2	3	16.7
Excluding *M. pyogenes* and/or *E. coli* and/or *Aerobacter aero.*									
Including *M. pyogenes* and/or *E. coli* and/or *Aerobacter aero.*	13	3	23.1	4	30.8	4	30.8	2	15.4
Total mixed infection	31	7	22.6	11	35.5	8	25.8	5	16.1

* Polymorphonuclear infiltration into bronchial wall; usually focal.
** Polymorphonuclear infiltration around and into bronchial wall with exfoliation of epithelial cells.
*** Polymorphonuclear infiltration around and into bronchial wall with exfoliation of epithelial cells, extensive involvement.

Discussion. The etiology of pulmonary infections complicating other diseases, particularly the respiratory diseases, has been repeatedly studied. Many reports[2,4,5,7,8,12,14-18] during the influenza pandemic of 1918–1919 indicated that bacterial pneumonia was frequently present among patients with influenza and that the predominant etiologic agent varied in the different areas studied. Prominent species were beta hemolytic streptococci, pneumococci (including strains which were then classified in group IV) and *Hemophilus influenzae*. Less frequently encountered but still important, were strains of *Staphyloccoccus aureus*. Finland[6] also described post influenzal staphylococcal pneumonia following a later epidemic in New England.

In 1947, Smillie and Duerschner[19] reported more specifically on the etiology of terminal bronchopneumonia, a condition more analogous to the one we have studied. They concluded that pneumococci, beta hemolytic strepto-

cocci and influenza bacilli were most frequent and that staphylococci were occasionally important. Previous studies by Whipple[20] and Cleveland[3] in postoperative pneumonia, and by Menten and co-workers[13] in secondary pneumonias of childhood had indicated the importance of the same four species. Menten's group, however, also included strains of *Streptococcus viridans* in their tables and a few patients infected with *B. mucosus capsulatus* and *B. pyocyaneus*. A few patients among those reported with post operative pneumonia also had *B. mucosus capsulatus (Klebsiella pneumoniae)* in their sputum.

In this study, among the 4 species recorded by other investigators as frequent causes of secondary bronchopneumonia, only the staphylococcus was found, even though the other species were especially sought. On the other hand, many Gram-negative rods among the genera Pseudomonas, Proteus, Aerobacter and Escherichia and, in addition, enterococci were found.

Liston[11] in a study of the etiology of bronchopneumonia in children, emphasized that organisms such as *H. influenzae* were frequently found in pure cultures early in the course and that they were rapidly replaced by other bacteria during the course of the disease the latter being of little importance. We do not feel that such a sequence of events occurred in our patients, since cultures of tracheal aspirates were obtained serially before the onset and in the early part of the course and the organisms found corresponded to those later found in the lung. The finding of the same organism in some patients in the blood stream antemortem and in the regional pulmonary lymph nodes at necropsy also substantiated the invasiveness of the bacteria. In addition, the correlation of pathologic evidences of infection with the occurrence of positive culture for the organisms reported here is striking and suggests an etiologic relationship.

Several reasons may be advanced for the observation that the infections in these patients were caused by bacteria other than the usual pulmonary pathogens. The most likely explanation is that antibiotic therapy inhibited the common pathogens, except staphylococci, therefore the more saprophytic species became predominant, and since the opportunity for invasion was ideal, they invaded the tissues in certain individuals. This has been suggested by the work of Barach[1] in patients with chronic pulmonary disease who were treated with antibiotics. Secondly, since these patients were studied in the summer, when the carrier rates of the usual pathogens were low, the more endogenous organisms may have been favored. Thirdly, the presence of a tracheotomy which bypasses the normal defense mechanism of the upper airway may have allowed direct implantation of the less pathogenic organisms into the lungs. The direct implantation of bacteria into the trachea by the suction catheter is a likely route of infection.

It should be emphasized that in addition to the evidence of infection almost all of these patients had moderate to extensive atelectasis. While this may be the most significant of the lesions present, and therapy should be against it, still this ample evidence of infection indicates that the latter was also important.

Summary. 1. Histologic examination of several blocks of tissue from each lobe of the lungs of 27 patients who died within 3 weeks of the onset of acute poliomyelitis revealed a high incidence of pulmonary infection. In all of the patients a tracheotomy and artificial respiration had been necessary.

2. Careful bacteriologic studies were made on the tracheal aspirations antemortem and the blocks of tissue used for histologic study in all patients.

3. Comparison of the histologic and bacteriologic findings indicated a good correlation between the occurrence of pulmonary infection of the bacterial type and the occurrence of bacteria in the cultures. The organisms involved were *Micrococcus pyogenes* var. *aureus*, enterococci, *Aerobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and species of Proteus.

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SUMMARIO IN INTERLINGUA

Pathogenese de Infectiones Pulmonar in Patientes de Poliomyelitis

1. In 27 patientes, morte intra 3 septimanas post le declaration de acute poliomyelitis, examines histologic de plure blocos de texito ab cata un del lobos pulmonar revelava un alte frequentia de infection pulmonar. In omne casos del serie, tracheotomia e respiration artificial habeva essite necessari.

2. In omne casos, exacte studios bacteriologic esseva executate super aspirationes tracheal de ante morte e super le blocos de texito usate in le examines histologic.

3. Per comparar le constatationes histologic e bacteriologic, nos establiva un bon correlation inter le occurrentia de infection pulmonar del typo bacterial e le occurrentia de bacterios in le culturas. Le organismos incontrate esseva *Micrococcus pyogenes* var. *aureus*, enterococcos, *Aerobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, e species de *Proteus*.

MECHANISM OF ACTION OF HEPARIN AS AN ETIOLOGICAL BASIS FOR HOMEOSTASIS (HEMORRHAGE AND THROMBOSIS)*

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Heparin in combination with a cofactor manifests two well established effects on the blood coagulation system: 1) interferes with the conversion (degradation) of prothrombin to thrombin, and, 2) blocks the action of thrombin. The mechanism by which such effects are produced has not been established. Investigators have conjectured that heparin with its cofactor inactivates the primary blood clotting component, that is, prothrombin[24], or rapidly neutralizes thrombin as it is produced during the process of conversion[5].

The anticoagulant effect of heparin is exhibited by: 1) a prolongation of the coagulation time of whole blood[10]; 2) a prolongation of the oxalated plasma clotting time obtained by the addition of thromboplastin and optimal calcium ion concentration, otherwise known as the "one stage procedure" or "thromboplastin-calcium ion clotting time"[5] ("Prothrombin Clotting Time")[24]; 3) an increase in the antithrombin activity of plasma by inactivating added thrombin[5].

Recent contributions to our knowledge of the mechanism of blood coagulation make possible an improvement in the formulation of the mode of action of heparin. A more sharply focused insight regarding the physiological role of heparin should enhance our understanding of the intricate problems of hemorrhage and thrombosis.

Materials. Blood obtained by venipuncture was used in this study. Human individuals were taken as a source of whole blood for *in vitro* heparin experiments. Dogs were used to investigate the *in vivo* heparin effect.

Plasma was separated by centrifugation from oxalated blood (0.1 molar sodium oxalate). Pooled, human, oxalated plasma was obtained from obstetrical patients with normal uneventful parturition on the first day postpartum. This clinical category was considered to be a more homogeneous group than nonhospitalized ambulatory patients. Plasma samples (2 to 8 hours old) from 4 to 10 patients were pooled for experiments on any given day. Such a procedure should minimize the effects of individual variation in blood clotting components, thus yielding a more reproducible sample.

In order to avoid misunderstanding and to insure clarity, biologically active reagents when used in blood coagulation studies

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should be carefully described and sources noted.

1. Heparin: a commercial preparation of high purity and unitage was used, sterile solution of "Heparin Sodium," 5,000 U.S.P. units per cc. prepared by the Abbott Laboratories, North Chicago, Illinois. Appropriate dilutions were prepared using saline as diluent. Concentrations are expressed in terms of heparin units rather than milligrams.

2. Ac-Globulin (Factor V): from bovine serum and plasma. Preparations from different laboratories were examined. Currently available information seems to indicate that the terms, "Ac-Globulin" and "Factor V" are synonymous[14] and they are regarded as such in this paper.

a) Bovine serum meticulously prepared according to the directions of Seegers *et al.*[12] was found to be a rich source of stable serum Accelerator Globulin. The advantage of such material is that it is readily available and easily prepared, and, it is a reagent used in a standard recommended procedure. Tests during the course of the present investigation revealed the presence of heparin cofactor and traces of thrombin in such prepared bovine serum.

b) Bovine serum Ac-Globulin, isolated and purified by Seegers as a lyophilized powder.

c) Bovine plasma Ac-Globulin concentrate, plasma fraction prepared from bovine plasma by Seegers as a lyophilized powder.

d) Bovine plasma Factor V, purified material obtained from F. Hoffmann-LaRoche Company, Basel, Switzerland. This preparation was a lyophilized powder of high purity and activity, free of Factor VII, prothrombin, fibrinogen and thrombin. The ultracentrifuge revealed a single prominent peak with a very minor one trailing. Electrophoretic analysis showed 2 prominent peaks.

3. Bovine serum Factor VII (Serum Prothrombin Conversion Accelerator). Purified material was obtained from F. Hoffmann-LaRoche Company, Basel, Switzerland. This material was a lyophilized powder. Ultracentrifuge and electrophoresis data revealed heterogeneity. However, the preparation was free of prothrombin, Factor V, fibrinogen and thrombin.

4. Thromboplastin: Two sources were used; rabbit brain prepared by the method of Brambel[6], and, bovine lung prepared by the method of Seegers *et al.*[12].

5. Fibrinogen, frozen, pooled, oxalated, human plasma was used as a source of this material. The cold fractionation procedure of Seegers[12] as modified by Lorand[16] was followed. Solutions of this material were found to contain 90% clottable protein.

6. Thrombin (Human and Bovine): Three preparations were studied from human and bovine sources. Two methods of prothrombin activation in the preparation of thrombin are included: activation with thromboplastin and calcium ions, and citrate catalysis where no thromboplastin and no calcium ions were used to produce thrombin from prothrombin.

a) Human Thrombin (thromboplastin [human placenta], calcium ion activation), prepared by the National Institutes of Health, Bethesda, Maryland, as a standard of reference having 22 units per mg. of dry material.

b) Bovine Thrombin (citrate catalyzed), prepared by Seegers having 600 units per mg. of dry material.

c) Bovine Thrombin (thromboplastin [bovine lung], calcium ion activation), high potency material (5,000 units per vial) prepared by Parke, Davis & Company, Detroit, Michigan, for investigative purposes was utilized in the routine tests. This preparation is a recommended reagent for the standard procedure for assaying prothrombin in plasma.

7. Protamine Sulphate, dry material was obtained from the Eli Lilly and Company, Indianapolis, Indiana.

Methods. Three well established standard blood clotting tests were utilized: 1) The coagulation time of whole blood (modified Lee-White[15] method) in uncoated glass tubes was measured to study the over-all effects of heparin. 2) Determination of the clotting time of test mixtures of plasma after the addition of selected unitage of thrombin (Thrombin Time) was used as an index to antithrombin (thrombin inhibitor) activity. The first appearance of fibrin strands was taken as the end point rather than the formation of a solid clot. 3) The two-stage procedure for assaying prothrombin[12] and the thromboplastin-calcium ion clotting time ("Prothrombin Clotting Time")[24] were used to explore the relationship of heparin to the various blood clotting factors.

The two-stage assay for prothrombin was used to determine the thrombin yield (in terms of units) under specifically defined conditions. The recommended procedure[12] requires the use of a reagent containing Ac-Globulin to obtain complete thrombin yield. In the present study this method was explored without supplementary addition of the accelerator. Such a procedure provided data with regard to Factor V deficiency and loss of its activity under experimental conditions as they pertained to heparin effects. This procedure was useful in ascertaining which

plasma component was inhibited or inactivated within the limitations of the method and our present state of knowledge.

The recommended unitage of thrombin (10 units per cc. of plasma) was used for defibrinogenation. The defibrinogenated plasma was incubated and prothrombin assays were run at hourly intervals.

Various agents, such as protamine and Factor V, were added (before and after defibrinogenation) as dictated by any given experimental pattern. Buffered saline (imidazole buffer pH 7.6) was used as solvent or diluent in all of the test procedures.

The summation rate effect of all coagulation factors present under any given experimental conditions was ascertained by changes in the plasma clotting time when rabbit brain thromboplastin and optimal calcium ion concentration were added. The method does not give specific data pertaining to the activity of any single clotting element. It is identified in this paper as the "thromboplastin-calcium ion clotting time" ("Quick[24] Prothrombin Clotting Time").

Two groups of experiments were set up: 1) clotting tests were run immediately after the constituents of the mixture were added; and, 2) the mixtures were incubated for varying periods of time at 25° to 30° C. and clotting tests were made at selected intervals. In the first group of experiments the immediate effects of the active components were observed. The second group presented evidence of continuing interaction of coagulation elements. Both groups of tests contributed significant data manifested by prolongation or decrease of the clotting time of plasma; or, increase or decrease of prothrombin unitage.

Results. Certain aspects of the work of previous investigators have been repeated and the results are presented here as a basis for comparison and continuity. The confirmatory evidence pertains to the effect of heparin added to oxalated plasma (convertibility of prothrombin) and the second phase (thrombin activity) of blood coagulation.

The relationship between plasma clotting time (thromboplastin-calcium ion clotting time), thrombin clotting time (using 10 units of thrombin per cc. of plasma) and heparin concentration (expressed as U.S.P. heparin units per cc.) is shown in Fig. 1. The tests were run immediately after mixing.

When the thromboplastin-calcium ion clotting time is ascertained on the heparin-plasma mixtures it is to be noted that the relationship of the clotting time to the anticoagulant concentration is not a straight line but a curve (Fig. 1.). The deviation from linearity suggests that multiple blood coagulation components are involved. These findings on their face value, do not reveal which primary clotting elements are affected.

For a selected unitage of thrombin, the clotting time of oxalated plasma increases as a linear function of the concentration of heparin within the limits of anticoagulant concentrations used. The slope of the line is determined by the unitage of thrombin used (Fig. 9). These data indicate that a thrombin inhibitor is produced immediately upon the addition of heparin to oxalated plasma confirming the reports in the literature[5,21]. A chemical substance is present in plasma which combines[7] with (or alters) heparin to give rise to a potent thrombin inhibitor (antithrombin). This component has been called "heparin cofactor." Heparin in the absence of the cofactor does not inhibit the action of thrombin. This property has been recognized by investigators for some time[7]. The combination of heparin and its cofactor has been designated as the "heparin-cofactor complex" because the nature of the chemical bonding is unknown at present. In view of the paucity of our knowledge, such terms are used only tentatively.

On the basis of the data presented in Fig. 1, it has been conjectured by other investigators that heparin with its cofactor inactivated prothrombin[24]; or, rapidly neutralized thrombin as it was produced during the process of

conversion[5]; or, that there was interference in some unkown manner with the conversion[5] of prothrombin to thrombin.

HEPARIN EFFECTS ON CLOTTING MECHANISM: FIRST PHASE. Incubation of heparin-plasma mixtures reveals a continuing reaction when the thromboplastin-calcium ion clotting time is run at 10-minute intervals (Fig. 2). Two effects are demonstrated: 1) For a given concentration of anticoagulant, a straight

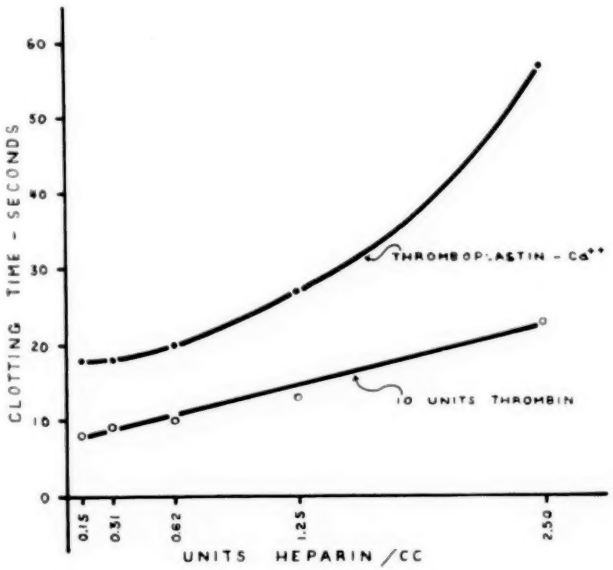

Fig. 1.—Human oxalated plasma: heparin effects.

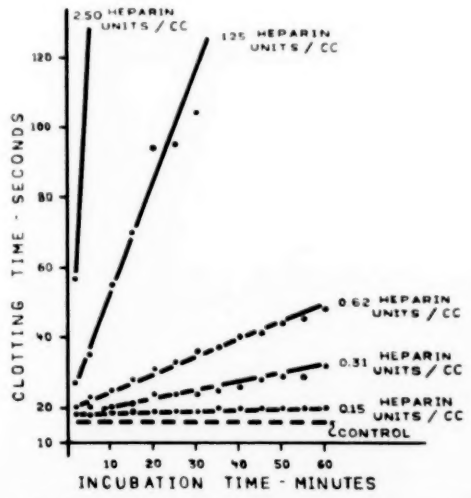

Fig. 2.—Human oxalated plasma: heparin antithrombin effect and progressive decrease in prothrombin convertibility. (Thromboplastin Ca++ clotting time.)

line relationship is observed between clotting time and incubation time compared to the curvilinear course obtained in Fig. 1. The slope of the line increases with increasing concentration of heparin. 2) The lines do not start at the origin which suggests the effect of a thrombin inhibitor that is produced initially. The progressive linear change along a slope implies continuing inactivation of a single clotting element. The evidence at this point does not identify which clotting element is involved because the procedure does not specifically measure any one component but a summation rate effect as already mentioned.

Associated with the disappearance of this coagulation factor in stored plasma, a concomitant increase in antithrombic activity[27] attributed to a heparin-like anticoagulant[21] has been described. These observations have a significant implication in the present study. It is common knowledge that addition of fresh plasma or Factor V to stored, oxalated plasma with prolonged thromboplastin-calcium ion clotting time immediately restores the clotting time to that of fresh plasma. Hence, the addition of heparin to fresh plasma may simulate and accelerate the production of progressive hypocoagulability correlated with decreased Factor V activity.

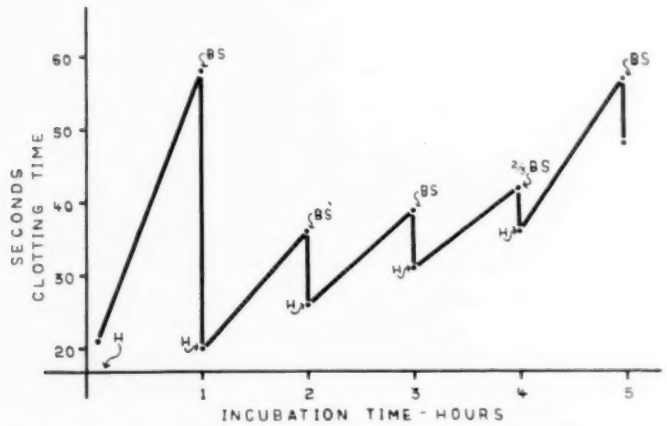

Fig. 3.—Human oxalated plasma: mutual reversibility-heparin effect and Ac-Globulin (Factor V) (Bovine Serum). (Thromboplastin Ca++ clotting time.)

The demonstration of a continued reaction as manifested by increasing hypocoagulability of the test plasma is of special interest. This same phenomenon is seen, but at much slower rate, when oxalated plasma is stored, namely, the disappearance or inactivation of what seems to be a labile component. This entity is sometimes identified as the "Labile Factor"[24] or more correctly named "Accelerator Globulin"[29] or "Factor V"[22].

The following experiment was devised to test the implication that the effect of added heparin in oxalated plasma results in progressive deterioration of Factor V and that mutual antagonism exists between these biochemical entities (Fig. 3). The thromboplastin-calcium ion clotting time was used in this experiment. The anticipated results were obtained.

Addition of serum or plasma Factor

V to heparinized oxalated plasma after one hour of incubation (using adsorbed bovine serum as a source of this component), or, purified bovine plasma Factor V results in immediate reversal of the heparin effect described above. The extent of the reversal depends upon the volume of the bovine serum used in the test or concentration of the purified component (Bovine plasma Factor V). There is an immediate decrease of the thromboplastin-calcium ion clotting time to the initial value of the heparinized plasma at the time of mixing, indicating that the missing coagulation factor was partially replaced. The purpose of this experiment was to add just enough Factor V to recover the initial clotting time of heparinized plasma (1.25 U.S.P. units Heparin per cc.) thereby making an approximate titer of the 2 agents. A larger volume of treated bovine serum or increased concentration of purified Factor V would have restored the clotting time to the pre-heparin value. When the same amount of heparin was again added, the thromboplastin-calcium ion clotting time was again progressively prolonged. This procedure of alternate addition of a source of Factor V and heparin to the same sample of oxalated plasma was repeated 3 times. In each case the same amount of bovine serum (10% of the test volume) (designated "B.S." in Fig. 3) and the same unitage of heparin (designated "H" in Fig. 3) was added. After each introduction of heparin the mixture was allowed to stand for one hour. The initial value of the thromboplastin-calcium ion clotting time after the first addition of heparin was not obtained following subsequent anticoagulant supplements. Correspondingly, the reversing effect of Factor V was also diminished with subsequent additions of the same volume of bovine serum. The inability to recover the anticipated clotting time was ascribed to the unavoidable dilution effect of the original plasma sample (repeated additions of volumes of heparin and adsorbed bovine serum).

To illustrate further the relative quantitative relationship, at the fourth step (Fig. 3) only two-thirds of the treated bovine serum volume were used and correspondingly the shortening of the thromboplastin-calcium ion clotting time was decreased. When the same amount of heparin (1.25 units per cc.) was again added, the slope was increased, showing greater effect of heparin compared to the amount of Factor V present.

This experiment also shows that sufficient heparin cofactor was present in the original plasma sample so that the coagulation inhibiting "heparin cofactor complex" was produced after successive additions of anticoagulant and its effects became manifested.

The cycle of alternate progressive prolongation of plasma clotting time with heparin and immediate decrease with Factor V is repeated a number of times in the same sample (Fig. 3). These data show that alternate addition of heparin and Factor V to the same sample of plasma results in mutual inactivation of these components. The effect of added heparin can be overcome by an excess of Factor V and *vice versa*. It is of some interest and importance that either agent can be added in excess of the other and exerts its own biological property. Such an experiment indicates that prothrombin is not the clotting component involved in the heparin effect. The stability of prothrombin is already well established by Seegers[29] and coworkers.

If purified Factor VII[1,14] is substituted for Factor V no effect on the clotting time of heparinized plasma is observed. If Factor V is added first and then followed with Factor VII a short-

er clotting time is obtained than that noted with Factor V alone. This observation indicates that Factor V and Factor VII are not interchangeable. It also shows the difference in behavior of these two accessory clotting elements. From the foregoing experiment it is seen that Factor VII is a "conversion rate determining component" only, and it is procedurally impossible to ascertain whether or not heparin and its cofactor have any effect on this entity. Factor V is an integral unit in the system for the conversion of prothrombin to thrombin. This experimental evidence contributes to our knowledge of the functions of Factor V and Factor VII.

The foregoing experiment shows that prothrombin itself is not altered as the result of the addition of heparin to plasma. The heparin effect is exerted on the conversion components (specifically, Facor V) that make up the system for the production of thrombin from prothrombin.

However, thromboplastin-calcium ion clotting time is only one of several types of coagulation tests which were used in this study. Such a procedure has been the subject of severe criticism. In general, it may be considered to be a nonspecific indicator of prothrombin convertibility, that is, any factors interfering with conversion components are expressed in terms of alteration of the plasma clotting time. We will now turn our attention to the results obtained with the two-stage clotting assay when heparin-plasma mixtures are incubated.

When thrombin is used for defibrinogenation as a preliminary step in the two-stage procedure there is a rapid decrease in the number of thrombin (prothrombin) units that can be recovered. It will be noted (Fig. 4) that for the same unitage, 3 different thrombins (Seegers' citrate catalyzed, National Institutes of Health standard human thrombin and Parke, Davis & Company Topical Thrombin) give diverse results. After one hour of incubation 50% of the original prothrombin units can be recovered using Bovine Topical Thrombin. The citrate catalyzed thrombin and human thrombin yield much lower values. Such a loss in convertibility of prothrombin has been attributed to a decrease in Ac-Globulin activity because of the instability of this component[30]. Addition of bovine serum as a reagent rich in Ac-Globulin activity results in complete recovery of prothrombin units regardless of the thrombin preparation used thereby attesting to the validity of the foregoing thesis.

Bovine thrombin (Parke, Davis & Company Topical Thrombin) was used in this series of experiments as a standard procedure (Figs. 5 and 6). As stated, the greatest decrease of prothrombin unitage occurred during the first hour of incubation when 50% of the original value was obtained, and remained constant for several hours thereafter. This property served as a useful reference for the study of heparinized plasma samples.

A rapid decrease in prothrombin unitage associated with loss of Factor V activity following defibrinogenation with thrombin has been described in the foregoing paragraphs. If bovine serum or protamine (Fig. 5) is added to plasma before defibrinogenation there is little change from the initial value in the subsequent recovery of prothrombin unitage. These results are interpreted as indicating that bovine serum contributed an excess of Factor V, and that protamine blocked or inactivated the agent responsible for destroying the prothrombin conversion accelerator. In this case, protamine may have interacted with a physiologically occurring heparin-like constituent.

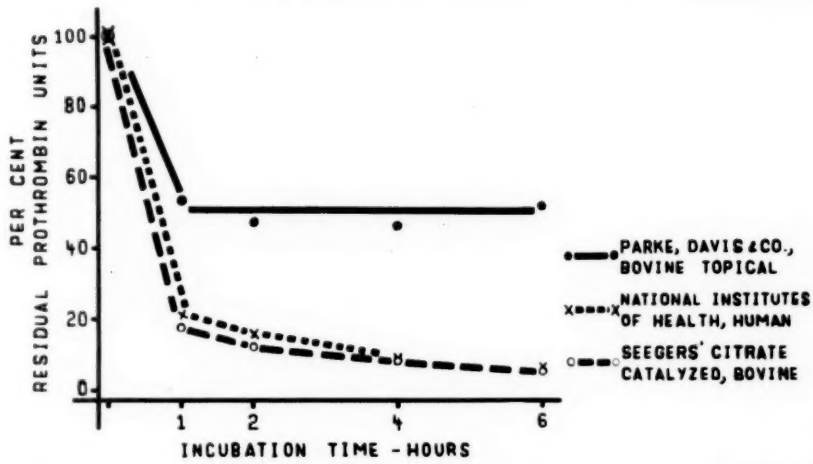

Fig. 4.—Human oxalated plasma: two-stage prothrombin assay. Comparison of three thrombin preparations. (Defibrinogenation with 10 units thrombin per cc. plasma.)

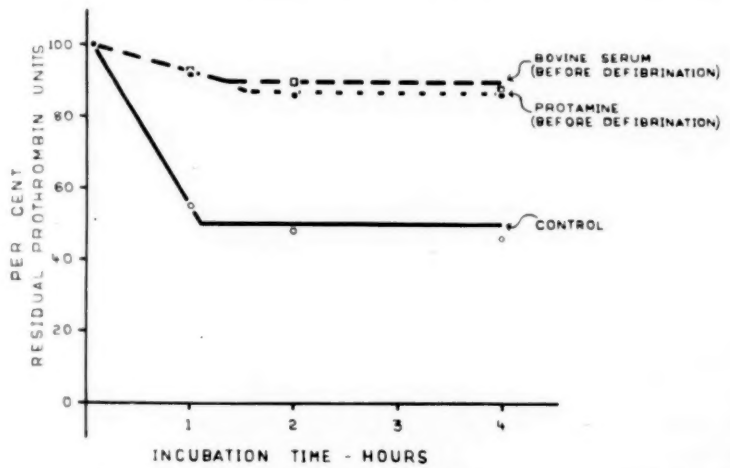

Fig. 5.—Human oxalated plasma: two-stage prothrombin assay. Sparing effect of protamine on Ac-Globulin (Factor V).

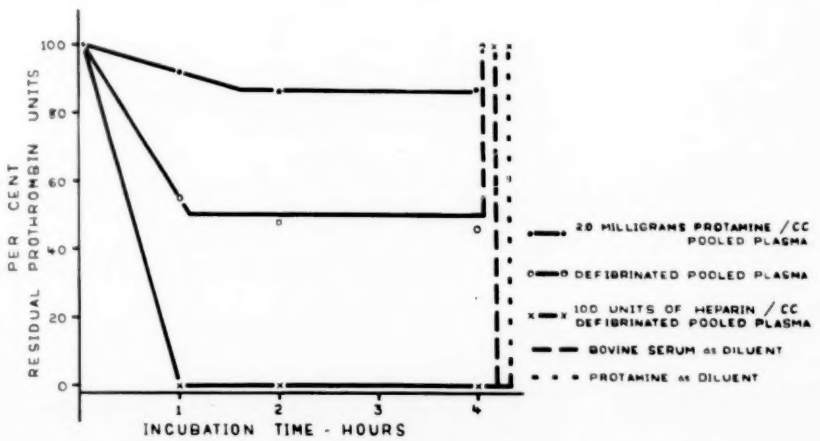

Fig. 6.—Human oxalated plasma: two-stage prothrombin assay. Neutralization of heparin effect-bovine serum and protamine.

sparing the activity of Factor V. Addition of protamine to defibrinogenated plasma after 4 hours' incubation had no effect on the decreased prothrombin unitage. It is construed that the interaction of the physiological heparin-like constituent with Factor V is irreversible under these conditions.

When a heparin concentration of 10 units per cc. of defibrinogenated plasma is used, it is to be observed from Fig. 6 that the prothrombin units decrease to zero at the end of one hour and remain at this level for a 4-hour period. It is presumed from these data that heparin cofactor is present in defibrinogenated plasma which is at variance with published reports[2]. If treated bovine serum or protamine is added at the end of the 4-hour period there is an immediate recovery of 100% prothrombin units. In the presence of protamine, these results indicate that the interaction of Ac-Globulin with a heparinized plasma constituent (heparin-cofactor complex) is reversible. With the addition of bovine serum, a new source of Factor V is supplied and prothrombin is again converted to thrombin. Heparin had not affected or altered prothrombin since it could be changed to thrombin if the proper piece was replaced in the conversion system. We note that a Factor V deficiency has been produced identical to that demonstrated previously when using the thromboplastin-calcium ion clotting time procedure to determine heparin effects.

It is seen from Fig. 6 that Ac-Globulin activity can be recovered from heparinized plasma deficient in this clotting component by the addition of protamine. This loss of activity is not due to the instability of this component but to a blocking effect. The blocking agent can be removed by interaction with protamine: in one case by preventing its action as shown when protamine is added to oxalated plasma before defibrinogenation (Fig. 5); or, as illustrated in Fig. 6, by reacting with the "Heparin-cofactor-complex" thereby liberating active Factor V. In both instances protamine was an active agent for increasing Factor V activity.

It is therefore reasonable to presume that the two inactive complexes of Factor V are identical. Since protamine is a specific heparin inactivator[11], the complexes would assume the nature of "Factor V-heparin-cofactor complex" combination. The chemical nature of such a linkage is unknown. On the basis of the data presented pertaining to the biological properties of the components involved it is presumed that the "Factor V-heparin-cofactor complex" under certain conditions can be split by protamine liberating active Factor V. No effect is observed, however, when protamine is added to incubated defibrinogenated plasma (without added heparin) manifesting loss in Factor V activity. The question of species differences in heparin arises, or, the difference between physiological heparin and a prepared material. The question as to why protamine can prevent loss of Factor V activity before defibrinogenation and not afterwards is an interesting subject for further study.

Having demonstrated that Ac-Globulin in treated bovine serum or as purified component reverses the heparin effect in plasma, we now turn our attention to the blood of a heparinized animal. Heparin (1,250 units) was administered to a 16.3 kg. dog by the intravenous route. Clotting times of whole blood, to which saline was added, were run at 15-minute intervals. Saline was added to the blood at the time blood was obtained because treated bovine serum (as source of Factor V) was added in another aliquot to test the effect of this component. The heparin curve is slightly lower than or-

dinarily obtained because of saline dilution.

When treated bovine serum is mixed with blood drawn at the peak of the heparin effect the coagulation time is decreased to preheparin values (Fig. 7). This experiment was repeated at intervals as shown on the graph. Within the limits of experimental error, all of the blood samples to which bovine serum had been added gave the same coagulation times regardless of the value obtained for untreated heparinized blood.

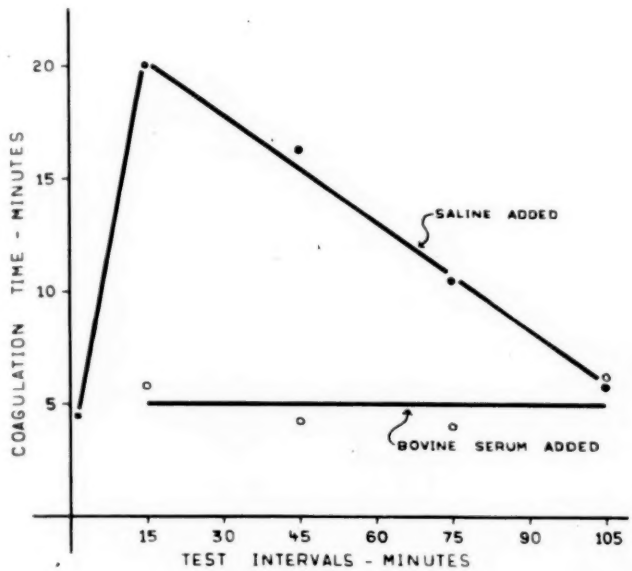

Fig. 7.—Heparinized dog. Effect of bovine serum *in vitro* on whole blood coagulation.

The antagonism of Factor V to the heparin effect is demonstrated using another type of blood clotting procedure. These results are identical with those obtained using protamine. The mechanism of the two reactions is entirely different even though the end result is the same, namely, restoration of the coagulation time of heparinized whole blood to normal. Protamine cannot be substituted for Factor V in the blood clotting mechanism.

HEPARIN EFFECTS ON CLOTTING MECHANISM: SECOND PHASE. The antithrombic effects present in plasma in relation to the properties of physiological and commercial heparin presents an intricate picture. Attention is focussed on the thrombin inhibitor effect of commercial heparin and on the general properties of the physiologically occurring component. No attempt has been made at this time to differentiate whether heparin destroys thrombin or interferes with the thrombin-fibrinogen reaction. In either case there is a loss of thrombin activity[13]. Four antithrombins[26] have been proposed to account for the multiple thrombin inhibitory properties of plasma. The relationship of these data to the concepts developed during the course of this investigation is under current critical study.

When heparin-plasma mixtures are

allowed to stand at room temperature and the thrombin clotting time tests are determined at intervals, it is to be noted (Fig. 8) that the thrombin inhibitor activity remains constant (one hour) for any given concentration of heparin. In the concentration range from 0.15 to 2.5 units per cc. the amount of antithrombin produced depends upon the unitage of the anticoagulant and remains unchanged for the duration of the experiment. These results indicate that the thrombin inhibitor is a stable biological complex and that a quantitative relationship exists between heparin and some component of plasma, that is heparin cofactor.

When heparin is added to plasma there is an increase in thrombin inhibitor effect proportional to the concentration of heparin. This finding is in agreement with a previously published report[25]. An experiment was devised to determine whether heparin in excess of cofactor could be added to plasma.

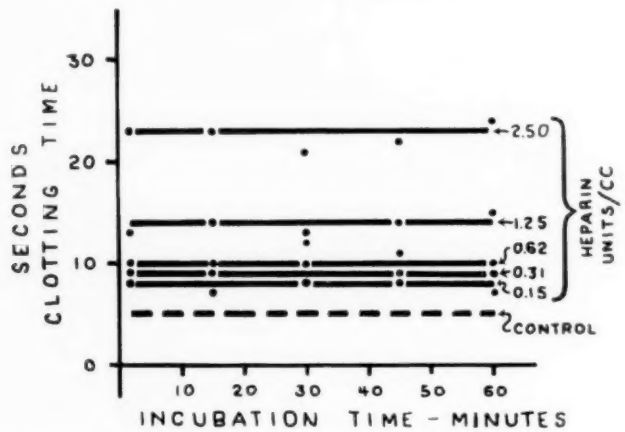

Fig. 8.—Human oxalated plasma: heparin antithrombin effect; 10 thrombin units/cc. plasma.

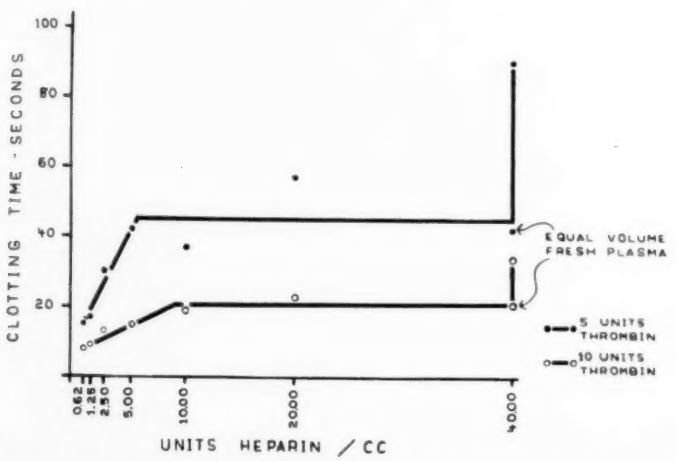

Fig. 9.—Human oxalated plasma: thrombin time. Demonstration of added excess of free heparin.

A limited amount of antithrombin was produced with increasing concentrations of heparin (Fig. 9). Addition of heparin after a given concentration is attained does not result in any further prolongation of the clotting time when thrombin is added. A wide range of heparin concentrations was studied (0.62 unit to 40.0 units per cc. plasma). A leveling off of the clotting time (which depends upon the thrombin unitage) occurred at 5 and 10 thrombin unit level indicating that all of the cofactor had been exhausted. Free heparin should be present in the mixture under such circumstances. To demonstrate the existence of free heparin, a fresh source of cofactor, as plasma, is introduced into the test mixture containing the greatest concentration of heparin. There is an immediate prolongation of thrombin clotting time indicating that more thrombin inhibitor has now been produced by the introduction of the sample of untreated plasma.

From this experiment it is seen that the heparin cofactor exists in plasma in finite amounts and can be exhausted by the addition of heparin to produce thrombin inhibitor (antithrombin). Since potentially active heparin cofactor can be demonstrated in plasma, it does not seem likely that free heparin can exist under such circumstances. In other words, free heparin cannot exist in normal circulating blood in the presence of free heparin cofactor.

The antithrombin effect of heparin in oxalated plasma can be nullified by the addition of thromboplastin or protamine. An antithromboplastin effect has been demonstrated with this anticoagulant by a number of investigators[3,4,8,9,10,17,19,20,31]. One investigator[23], however, claims that thromboplastin does not inactivate heparin. A survey of the literature also reveals that there is an increase in antithrombic activity of stored plasma[27] due to a heparin-like anticoagulant[21] which can be counteracted with protamine[28]. The results of the present study indicate that serum and plasma Factor V likewise have this same property.

The comparative antiheparin effects of rabbit brain and bovine lung thromboplastin and treated bovine serum are illustrated in Fig. 10. The thrombin times are plotted against varying volume per cent counter-active (3.12 to 50% by volume in heparinized [0.625 units per cc.] oxalated plasma). Five units of thrombin per cc. of plasma were used to clot the mixture. It is to be noted that rabbit brain thromboplastin had the least effect on the thrombin clotting time, while bovine lung thromboplastin and treated bovine serum had nearly identical potency of appreciable magnitude. The similarity of effect of these 2 reagents in this clotting system indicates the existence of another antithrombin inhibitor, namely, Factor V. Thromboplastin is not a gross contaminant of treated bovine serum as determined by critical testing.

The effect of treated bovine serum is again illustrated in Fig. 11 where the antithrombin effect of heparin (produced by 0.625 unit heparin per cc. oxalated plasma) is maintained for one hour. Addition of an equal volume of this reagent to the anticoagulated plasma immediately restores the thrombin clotting time to the preheparin level. Addition of more heparin (the same unitage as initially) to such antithrombin inactivated plasma again results in the production of more thrombin inhibitor. Since the original heparinized plasma thrombin clotting time is not recovered by the addition of the same amount of anticoagulant, it is presumed that an excess of antithrombin inhibitor was introduced by the equal volume of treated bovine se-

rum. These results are interpreted as signifying that serum Factor V and heparin thrombin inhibitor (antithrombin) are mutually antagonistic. The quantitative relationships are unknown because purified components are yet to be prepared.

V per cc. Similarly, when the same amount of bovine plasma Factor V was added to oxalated plasma followed by 0.625 unit of heparin only one-third of the heparin effect (as thrombin clotting time) was obtained. It is presumed that either Plasma Factor V

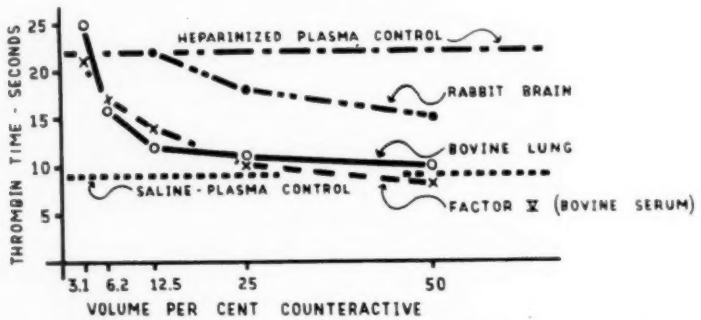

Fig. 10.—Antithrombin (heparin effect) inhibition by thromboplastin and Factor V (Bovine Serum); 0.625 unit heparin and 5 units thrombin per cc. plasma.

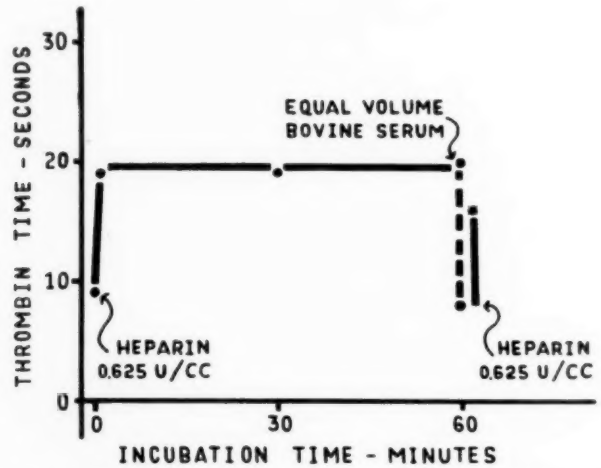

Fig. 11.—Human oxalated plasma: mutual reversibility: heparin effect-Ac-G(Factor V) (Bovine Serum); 5 units thrombin per cc.

Purified bovine plasma Factor V was substituted for bovine serum (Fig. 12) to study its effect as an antithrombin inhibitor. Only partial reversal of the heparin effect (produced by 0.625 unit heparin per cc. of plasma) was possible with 3.8 mg. of bovine plasma Factor

behaves differently from Serum Factor V and is not as potent as an antithrombin inhibitor, or, that the Plasma Factor V preparation contained Serum Factor V. These results are equivocal since such a large concentration of Plasma Factor V was necessary to ob-

tain a significant reversal of the heparin effect in plasma. A number of questions need to be considered. According to published reports[30] this plasma component may be an inert protein, and can be changed to the serum entity by the action of thrombin.

A dual function for Factor V has been developed: 1) as an essential component in the conversion system of prothrombin[22] to thrombin; and, 2) as an antithrombin inhibitor. In this role it antagonizes either the physiologically occurring heparin-like complex, or, added heparin in the presence of its cofactor. On the other hand, in pure component systems, Seegers[30] has shown that thrombin destroys Factor V. This intricate relationship and competitive inhibition needs to be clarified further.

Discussion. An integration of the foregoing experimental data is presented as a tentative generalized schema of the physiological blood coagulation system (Fig. 13). A rein-

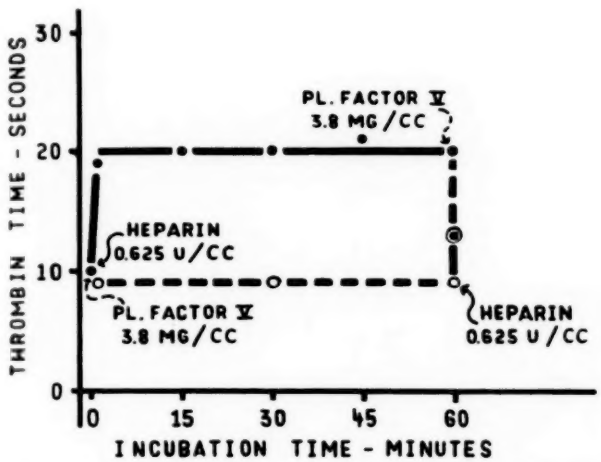

Fig. 12.—Human oxalated plasma: mutual reversibility: heparin effect-Factor V (plasma fraction); 5 units thrombin per cc.

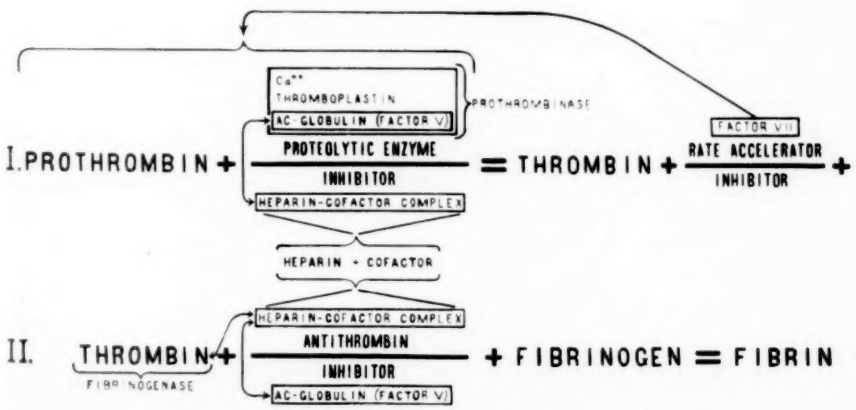

Fig. 13.—Tentative schema: physiological blood coagulation system.

terpretation of the clotting mechanism in the light of the biological significance of coagulation inhibitors appears justified. The blood coagulation system is poised in favor of clotting as a normal protective physiological mechanism. Blood clotting is visualized as a series of specific proteolytic enzyme reactions and stoichiometric interactions between components. Resolution of this intricate pattern of protein interrelationships is no longer in the realm of impossibility.

For purposes of discussion, the convention of dividing the blood coagulation mechanism into two phases is utilized. Clotting does not occur as two separate reactions, that is: 1) production of thrombin from prothrombin, and, 2) clot (fibrin) formation by the reaction of thrombin with fibrinogen. These processes may occur consecutively, but may also proceed simultaneously. These two reactions may be interconnected by entities that can govern both of them.

A characteristic property is imparted to the environment when heparin and its cofactor are present. The differentiation between chemical combination, or alteration between constituents, cannot be made with any degree of certainty at this time. Until evidence is produced to the contrary, a combination is presumed and the term "heparin-cofactor complex" is coined to denote biochemical characteristics of plasma after interaction of heparin with its cofactor. This entity has properties peculiar to itself: inhibitor of Factor V, thromboplastin, and thrombin. From the foregoing presentation of the data, the existence of free heparin cofactor has been demonstrated. It is not likely that heparin can exist in the free state in the blood stream for reasons already given.

The physiological heparin-cofactor complex can be inactivated by protamine before but not after defibrinogenation with thrombin. Conversely, protamine inactivates this complex produced after the introduction of commercial heparins to blood or oxalated plasma with the result that Factor V is again available for prothrombin conversion. The question of species specificity and chemical structure of physiologically occurring constituents versus isolated purified components, needs to be considered.

In Reaction I it is to be noted that prothrombin is degraded to thrombin by a proteolytic enzyme whose activity is governed by an inhibitor. The "Proteolytic Enzyme" is defined as a "Prothrombinase" made up of three essential components: Factor V, thromboplastin and calcium ions in proper proportions. Variations in concentration of any one of these three essential units result in variable thrombin yield[18]. The definition of such a tripartite enzyme deviates somewhat from the usual interpretation.

The "Inhibitor" in this case is defined as the "Heparin-cofactor Complex" which is presumed to be a product of the interaction of heparin and its cofactor. The physiologically occurring heparin-like complex quantitatively inactivates Factor V, and does not interfere with prothrombin. The rate of this inactivation is a slow process in oxalated plasma and a rapid reaction following defibrinogenation with thrombin. Heparin with its cofactor affects the activity of "Prothrombinase" by inhibiting one of the essential pieces of the enzyme.

When thrombin is produced another entity appears, namely, Factor VII (S.P.C.A.). The present experimental study indicates that some such entity is produced during the degradation reaction of prothrombin. It may be a fragment of prothrombin (derivative) or altered prothrombin. The function of

Factor VII can be demonstrated to be that of a "Rate Accelerator" in speeding up the degradation of prothrombin. The number of thrombin units is not altered but they are produced faster. Factor VII cannot be interchanged with Factor V, they are separate and distinct entities. An "Inhibitor" is indicated for Factor VII but its properties as yet have not been clearly defined.

In Reaction II, thrombin is defined as a proteolytic enzyme designated as "Fibrinogenase" whose specific substrate is fibrinogen. The end products of this reaction are fibrin and fibrinopeptide[16b]. Thrombin activity is governed by an "Antithrombin," which in the present case is defined as "Heparin-cofactor Complex." Diverse types of antithrombins have been described in the literature[26].

The "Antithrombin" in turn is governed by an "Inhibitor," which in Reaction II is defined as Factor V. Thromboplastin also inactivates this antithrombin (heparin-cofactor complex) (Fig. 10).

Factor V and heparin-cofactor complex stoichiometrically inactivate each other in both reactions (Fig. 13). Heparin-cofactor complex quantitatively inactivates thrombin. There is a competitive reaction between Factor V and thrombin for heparin-cofactor complex.

There are three components that possess dual roles in the blood coagulation system: heparin with its cofactor, Factor V, and thromboplastin. Heparin with its cofactor inactivates Factor V by forming a "Heparin-cofactor-Factor V complex" thereby producing a deficiency in the prothrombinase system which results in decreased thrombin production. It creates a plasma Factor V deficiency. Here lies the explanation for the defect in prothrombin conversion ascribable to heparin. Factor V and thromboplastin are essential components for the conversion of prothrombin to thrombin. They can also serve as antithrombin (heparin-cofactor complex) inhibitors in a quantitative relationship.

From the schematic diagram presented it may also be possible to forecast conditions under which intravascular thrombosis may occur. If physiological production of heparin and cofactor are diminished and excess Factor V produced, or if it remained constant, then, at the site of any peripheral vascular trauma, with resulting liberation of tissue juices rich in thromboplastin locally, a thrombus could form at the locus.

Similarly, Factor V deficiency with hemorrhagic complications[22] is a known clinical entity. The etiological factor involved may be a physiological increase in heparin and its cofactor, rather than a real deficiency of the prothrombin conversion component. A re-evaluation of such cases in the light of the present findings should prove of interest.

Conclusions. 1. A quantitative relationship exists between heparin and its cofactor.

2. Heparin with its cofactor: a) inactivates serum and plasma Factor V; b) but does not inactivate prothrombin.

3. A mutual quantitative inhibitory relationship exists between heparin-cofactor complex and Factor V, thrombin and thromboplastin.

4. The interactions between these particular components are stoichiometric rather than enzymatic.

5. The blood clotting system is composed of enzymatic and stoichiometric protein interactions.

6. Free heparin cofactor and free Factor V have been demonstrated in oxalated plasma.

7. Under the conditions mentioned in 6, free heparin cannot exist in circulating blood.

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(Interlingua on page 298)

RETROGRADE CONDUCTION OF VENTRICULAR EXTRASYSTOLES TO THE ATRIA

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It has been well known that impulses can travel in either direction along nerve and muscle fibers. Similarly, it has been well established that retrograde conduction in the heart occurs with both ease and frequency in lower animals. Thus, in certain fish reverse conduction in the heart is common and may take place with greater speed than orthograde conduction[3,10]. In the frog, this reversal of conduction was first observed in 1850. Skramlik found this to be true only under certain conditions and concluded that the speed of conduction was faster in the orthograde manner[11].

In mammalian hearts and particularly in man, it has been generally accepted that retrograde conduction of ventricular extrasystoles to the atria or sinus node is the exception[1,4,7,8]. The first convincing example of ventricular extrasystoles with retrograde conduction was published in 1912[5]. Since then, only a few single instances have been reported.

It is difficult to decide by means of standard electrocardiographic leads whether a ventricular extrasystole is conducted retrograde or not for the P waves are usually invisibly hidden in the QRS complex of the extrasystole. The value of the esophageal lead for this purpose was emphasized by Brown[2] in 1936. In 1951, Kistin and Landowne[8], utilizing the esophageal lead, reported that 15 of 33 subjects revealed retrograde conduction of ventricular extrasystoles to the atria. They concluded that this was a common occurrence in the human heart. Because these results contradicted previous findings and opinions we decided to re-study this problem.

Method. The subjects for this investigation were hospitalized patients whose electrocardiograms exhibited sinus rhythm with ventricular extrasystoles. Age, sex, pathology or medication were without influence on choice. The Nyboer esophageal tube with multiple metal rings (electrodes) at various levels was employed. A twin-channel Sanborn Visocardiette machine was used and standard limb lead II was simultaneously recorded. The esophageal lead was unipolar and recorded at the level of the atria as determined by the registration of a diphasic P wave with initial positivity and a deep Q wave followed by an R wave.

The criteria used for the determination of retrograde conduction were similar to those of Kistin and Landowne: 1) The P′ (where P′ is the atrial wave caused by the retrograde conduction to the atria) waves may be different in contour from those of the sinus P wave; 2) The P′ wave may be premature in regard to the sinus P wave and then the succeeding P′–P interval is longer

than the sinus P–P interval; 3) The presence of fusion P waves (where part of the atrium is activated by the sinus node and part by the retrograde impulse originating from the ventricular extrasystole, resulting in a fusion P wave whose contour is composed of characteristics of the normal sinus P wave and the retrograde conducted P′ wave).

Some problems arose from these seemingly simple criteria, chief of which was the interpretation of the P–P′ and P′–P intervals when sinus arrhythmia was present. This was particularly true when the differences between sinus P intervals were equal to or greater than the differences between the P–P′ intervals. Also the evaluation of changes in the contour of the P waves was at times difficult because of two factors. The first was the superimposition of the P wave on the QRS or T of the normal sinus complex. This caused the P wave to be modified even in those cases in which there was absolute proof that retrograde conduction was not taking place. Secondly, the sinus P preceding a QRS complex would occasionally change in contour due to respiratory movements of the heart which would inadvertently alter the relationship between the esophageal electrode and the atrium (Fig. 1). That such changes were due to respiratory movements was confirmed by having the patients cease breathing, thus causing abolition of the changes in the contour of the normal sinus P wave. Hence, the value of this criterion was limited unless the changes were striking or similar changes were not observed in the normal sinus P waves. The one criterion which we considered to be most significant was the prematurity of the P′ wave.

Results. Thirty-five subjects were studied. Their ages varied fom 39 to 87 years, the average being 58 years. Twenty-nine were male and 6 were female. Two main categories were found.

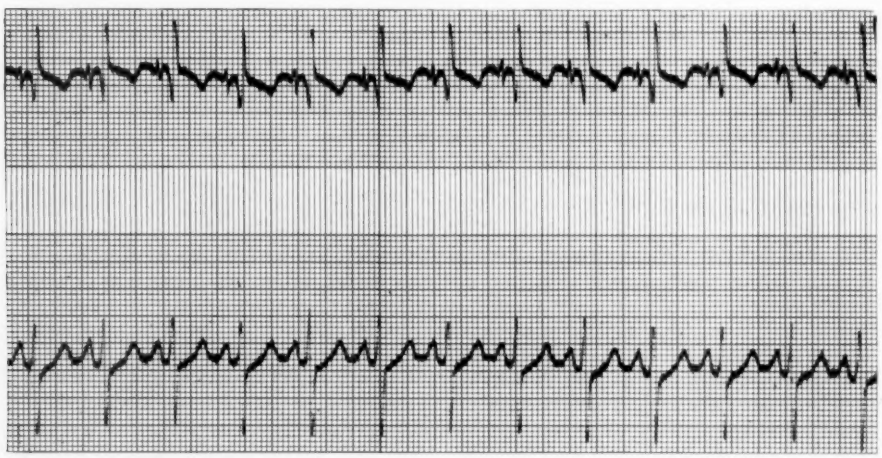

Fig. 1.—A demonstration of the various configurations of the P wave during sinus tachycardia caused by the altering relationship of the esophageal electrode to the atrium due to respiratory movements.

1. INDETERMINATE. *A. Sinus tachycardia.* There were twenty-five (71%) in this group. The average sinus rate was 95 per minute. Here the tachycardia was so rapid that normal atrial activation following the ventricular extrasystoles appeared before retrograde conduction could take place (Fig. 2). In other words, the ventricular extrasystoles appeared so late in diastole (in relation to the total diastole) that the atria were already activated by the sinus node. If retrograde conduction were possible it would have reached the atria and sinus node during

their refractory period, thus preventing conduction. How many of the ventricular extrasystoles of this group could have been retrograde-conducted, providing the sinus rate were slower, is only conjectural at this time. Therefore this group cannot be evaluated.

B. Sinus arrhythmia. One (3%) composed this group, which could not be accurately assessed because of marked sinus arrhythmia and the absence of indisputable changes in the contour of the P waves.

2. DETERMINATE. *A. Ventricular extrasystoles which are retrograde conducted.* There were eight

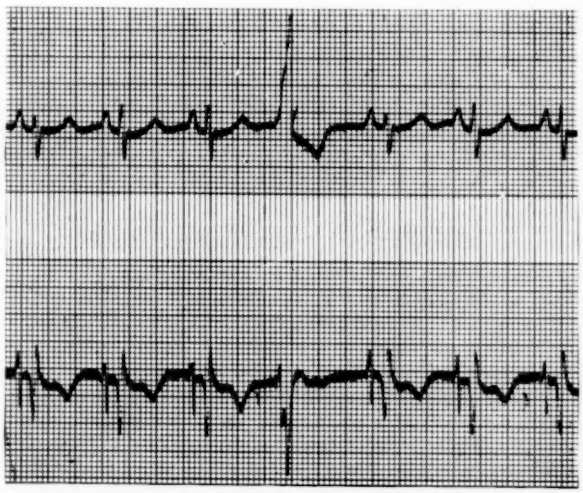

Fig. 2—Sinus tachycardia causing normal atrial activation to occur before retrograde conduction is possible. Notice the sinus P wave at the onset of the ventricular extrasystole.

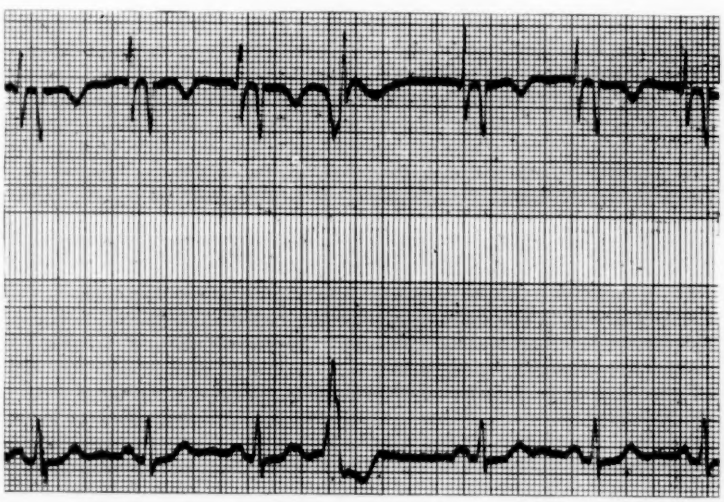

Fig. 3.—An example of retrograde conduction of a ventricular extrasystole. Notice the prematurity of the P′ wave, prolongation of succeeding P′–P interval and the slight change in contour of the P′ wave.

(23%) unquestionably in this group. These were manifested by prematurity of the P′ wave and prolongation of the following P′–P interval over that of the P–P interval with or without accompanying changes in the configuration of the P′ wave (Fig. 3). The average sinus rate was 72 per minute and the interval from the beginning of the QRS complex to the intrinsic deflection of the P′ wave ranged from 0.12 to 0.18 of a second. This was well within the acceptable values for retrograde-conducted P′ waves[8]. For a summary of the pertinent facts of this group see Table 1.

TABLE 1.—VENTRICULAR EXTRASYSTOLES WHICH ARE RETROGRADE CONDUCTED

Patient	*Ventricular Rate*	*Interval in Seconds*			
		P–P	*P–P′*	*P′–P*	*Q–P′*
		.82	.74	.92	
FT	73	.80	.72	.96	.18
		.72	.68	.78	
CA	79	.70	.66	.80	.12
		.84	.70	1.04	
LM	68	.86	.70	1.02	.14
JM	71	.84	.72	.96	.14
NM	75	.82	.76	.92	.13
CH	75	.80	.64	1.10	.16
IB	71	.82	.80	.82	.18
FB	60	1.00	.84	1.04	.16

This table summarizes the pertinent findings of those ventricular extrasystoles which were conducted retrograde to the atria. See text for explanation.

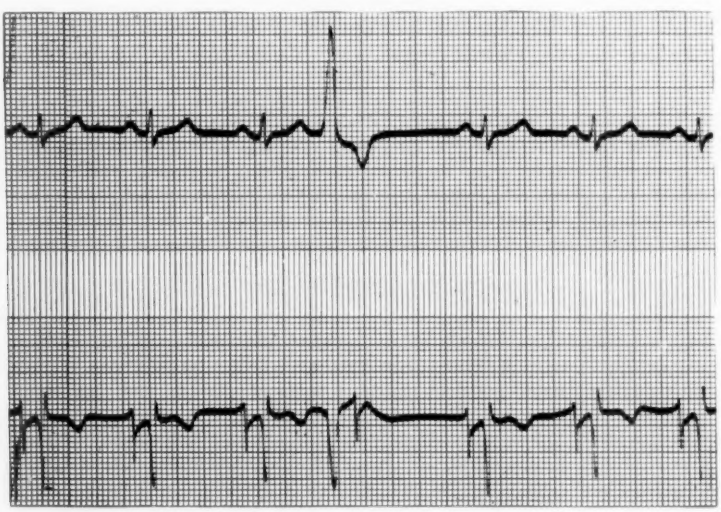

Fig. 4.—An example of a ventricular extrasystole not conducted retrograde and without interference from sinus tachycardia or sinus arrhythmia. The P wave occurs 0.12 second after the onset of the QRS complex which is a sufficient interval to permit retrograde conduction to manifest itself if present.

B. Ventricular extrasystoles which are not retrograde-conducted and suffer no interference from the sinus node. This category consisted of one case (3%). The sinus rate was 75 per minute, which was sufficiently slow to permit retrograde conduction to manifest itself if such were the situation (Fig. 4).

Discussion. The conditions under which a retrograde impulse encounters excitable or refractory atrial tissue depend upon the sinus rate (the higher the rate the less chance for excitable atrial tissue to exist when the retrograde impulse arrives), the length of the refractory period of the atria[9], the delay of passage through the A–V node[6] and the length of coupling of the ventricular extrasystole (the longer the coupling interval the greater the opportunity for the sinus node to discharge and to have the atria in a depolarized and therefore refractory state at the time when the retrograde impulse would arrive).

Analysis of our results shows that 74% of the subjects studied were in the indeterminate category due either to sinus tachycardia or sinus arrhythmia. Of the 26% that could be evaluated, 89% had retrograde conduction to the atria. This percentage is considerably higher than has been generally considered to prevail. Also it should be appreciated that only one out of the 9 subjects investigated who had the suitable conditions for the occurrence of retrograde conduction failed to exhibit this phenomenon. The full significance of this must be tempered by the fact that there is no means of knowing how many of the indeterminate group would not have been retrograde-conducted providing favorable conditions prevailed.

Thus the sinus rate apparently is the single most important factor in determining whether or not a ventricular extrasystole will be conducted retrograde. The higher the rate the less likelihood of retrograde conduction occurring. This is illustrated in this study by the fact that when the average sinus rate was 95 per minute (Indeterminate category, group A) none of these had ventricular extrasystoles which were retrograde-conducted. Similarly when the sinus rate averaged 72 per minute (Determinate category, groups A and B) 8 of 9 were retrograde-conducted.

Hence, we believe that if the sinus rate is 75 to 80 per minute or lower, most ventricular extrasystoles will be retrograde-conducted to the atria. Since in the human heart ventricular extrasystoles are most common when the lower sinus rates prevail, we believe that retrograde conduction is a fairly common event.

Summary. With the aid of esophageal leads the incidence of reversed conduction of ventricular extrasystoles to the atria was investigated in 35 subjects. In 25 of these patients a sinus tachycardia prevented the determination of the presence of retrograde conduction. The latter was found in 8 of 9 patients with ventricular extrasystoles and a slow sinus rhythm.

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SUMMARIO IN INTERLINGUA

Conduction Retrograde del Extrasystoles Ventricular Verso le Atrios

Per medio de derivationes esophagee le frequentia de conduction retrograde de extrasystoles ventricular verso le atrios esseva investigate in 35 subjectos. In 25 casos un tachycardia sinusal preveniva le identification de conduction retrograde. Sed un tal esseva constatate positivemente in 8 inter 9 patientes con extrasystoles ventricular e un lente rhythmo sinusal.

SUMMARIO E CONCLUSIONES IN INTERLINGUA

(*See page 264 for original article*)

Chlorido Cobaltose in le Tractamento de Certe Anemias Refractori

Chlorido cobaltose esseva administrate per via oral a 8 patientes con chronic anemia del typo normocytic e normochromatic. Le gruppo includeva 4 patientes con chronic glomerulonephritis, 1 con chronic pyelonephritis, 2 con chronic arthritis rheumatoide, e 1 con morbo de Hodgkin. Esseva observate in omnes un augmento del hematocrito, del conto de erythrocytos, e del hemoglobina.

Le administration diurne de inter 0,12 e 0,16 g chlorido cobaltose administrate in doses dividite se monstrava adequate pro le obtention de responsas satisfactori e pro le mantenentia del assi attingite nivellos hematologic.

Le effectos lateral esseva minime. Illos consisteva in leve disturbationes abdominale e in nausea de grado equalmente leve. Ambes esseva facile a regularisar per augmentar le doses gradualmente.

Cobalt pare esser un droga de valor in tractar anemias secundari a morbos chronic.

CONCLUSIONES IN INTERLINGUA

(*See page 276 for original article*)

Le Mechanismo del Action de Heparina Como Base Etiologic de Homeostase (Hemorrhagia e Thrombosis)

1. Il existe un relation quantitative inter heparina e su cofactor.
2. Heparina insimul con su cofactor inactiva le factor V del sero e plasma sed illo non inactiva prothrombina.
3. Un relation quantitative de inhibition reciproc existe inter le complexo de heparina plus cofactor e factor V, thrombina, e thromboplastina.
4. Le interactiones del componentes mentionate es stoichiometric plus tosto que enzymatic.
5. Le systema de coagulation sanguinee consiste de interactiones enzymatic e stoichiometric de proteinas.
6. Cofactor de heparina e factor V in stato libere ha essite demonstrate in plasma oxalate.
7. Sub iste conditiones, *i.e.*, le conditiones mentionate in paragrapho 6 supra, heparina in stato libere non pote exister in sanguine circulante.

THE NEPHROTIC SYNDROME AND CHRONIC HYPOTENSION ASSOCIATED WITH PRIMARY SYSTEMIC AMYLOIDOSIS

REPORT OF A CASE

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The nephrotic syndrome in adults is so frequently identified as an aspect of chronic glomerulonephritis that the possibility of its association with other conditions often is overlooked. Renal amyloid disease secondary to chronic suppuration (tuberculosis, bronchiectasis, osteomyelitis or empyema) or to granulomatous processes (chronic rheumatoid arthritis, multiple myeloma, tertiary syphilis) may elicit a nephrotic syndrome in which the primary lesion usually is easily recognized. On the other hand, primary systemic amyloidosis, rare in itself, is uncommonly associated with the nephrotic state: only 9 instances were described in a recent analysis of 50 cases[1]. In such a situation the diagnostic difficulty is increased by absence of the primary causes accounting for secondary amyloidosis.

A patient with generalized primary systemic amyloidosis primarily manifested by the nephrotic syndrome and chronic hypotension was recently seen. The diagnosis was not established despite extensive examination, until amyloid was seen in a biopsy specimen from the cervix uteri. It has been suggested that ACTH should be given in every case of amyloidosis[1], no improvement was noted in this patient during the administration of both ACTH and cortisone, and death occurred 11 months after onset of symptoms.

Case Report. The patient was a housewife, aged 47 years, who was first admitted to the Cleveland Clinic Hospital on September 27, 1954, with complaints of extreme weakness, pain in the right leg and lower back, loss of weight, and severe constant headaches, of approximately 9 months' duration. She had been in good general health except for an attack of trigeminal neuralgia in 1947 and an uncomplicated cholecystectomy in 1952. There was no history of allergy. The family history disclosed that her mother and one brother had died of heart disease and her father of abdominal carcinoma.

Nine months prior to admission, an intermittent aching pain in the right leg appeared and radiated into the lower back. Five months before entry, she noticed swelling of both lower legs, of hands and face, and she was hospitalized elsewhere for 55 days. Proteinuria and microscopic hematuria were noted for the first time. There was a gradual loss of appetite with marked muscle wasting and a loss in weight of 20 pounds. A diagnosis of subacute glomerulonephritis seemed reasonable; cortisone, 200 mg. daily for 12 days, followed by 40 units of ACTH gel every 3 days for 3 weeks was given without improvement. She was discharged from the hospital on a high-protein, low-salt diet. During the following 4 weeks, light-headedness became so marked that she was unable to walk alone more than a few steps. Difficulty in swallowing was noted at about that time. She frequently had nausea and vomiting. She became progressively weaker at home and was brought here.

Physical examination at the time of admission revealed the patient's temperature to be 97° F., pulse rate 80, respiratory rate 20, and the blood pressure 82/46 mm. Hg lying and 54/0 mm. Hg standing. She was unable to sit up in bed without becoming dizzy. Generalized muscular atrophy was marked; she could not maintain a hand grip or raise her extremities for more than a few seconds. Several small purpuric lesions were present on the face and the neck. The right pupil was larger than the left; there was slight bilateral eyelid lag. The fundi were normal. The tongue was not enlarged. There was no lymphadenopathy and the thyroid could not be palpated. The neck veins were not distended. There was normal resonance on percussion of the thorax; a few medium and coarse rales were heard on auscultation over the mid-portion of the right lower lobe posteriorly. The heart was normal except for a soft systolic murmur at the apex which was not transmitted. No masses were felt in the abdomen, but there was moderate tenderness in both costovertebral regions. There was 2-plus pitting pretibial, ankle and sacral edema, and slight puffiness of the hands and face. Neurologic examination was within normal limits except for slightly diminished reflexes.

Laboratory data are summarized in Tables 1, 2, 3, and 4. In addition, roentgenograms of the chest revealed a normal-sized heart; the lung fields were clear. A skull film showed normal calvarium and sella turcica. An intravenous urogram showed prompt excretion and concentration of the opaque medium by both kidneys; the calyces were normal in shape and there was no evidence of ureteral obstruction. A roentgenogram of the esophagus was normal. An electrocardiogram showed sinus tachycardia, low voltage of the QRS complexes, and slight prolongation of the Q–T interval suggestive of hypocalcemia or hypopotassemia. All hematologic studies were normal except for a persistent leukocytosis and elevation of the sedimentation rate. Ex-

TABLE 1.—URINALYSES

		This Patient			
	Normal	*July 1954*	*September 1954*	*October 1954*	*November 1954*
Proteinuria (gm./12 hr.)	0–0.2	7.2	7.2	6.4	7.4°
Hematuria (millions/12 hr.)	0–0.500	1.530	0.160	3.331	17.600
Cylindruria (millions/12 hr.)	0–0.009	—	0.016	0.030	0.077
Bence-Jones protein	absent	—	absent	—	—
Urine porphyrins	negative	negative	negative	—	—
17-ketosteroids, female (mg./24 hr.)	4–7	—	4.2	—	—

°Daily average.

TABLE 2.—ELECTROPHORETIC PATTERNS OF SERUM PROTEINS

			This Patient							
	Normal		*July 1954*		*September 1954*		*October 1954*		*November 1954*	
	gm./100 cc.	*%*	*gm./100 cc.*	*%*	*gm./100 cc.*	*%*	*gm./100 cc.*	*%*	*gm./100 cc.*	*%*
Total	7.44		4.60		3.24		3.52		3.76	
Albumin	4.74	63.5	—	—	1.00	31.1	1.22	34.4	1.29	34.4
Alpha-globulin	0.54	7.2	—	—	α1, 0.34	10.5	1.18	33.6	0.90	23.8
					α2, 0.86	26.4				
Beta-globulin	1.11	15.1	—	—	0.87	26.7	0.91	25.8	1.25	33.2
Gamma-globulin	1.04	14.1	—	—	0.17	5.3	0.21	6.2	0.32	8.6

amination of the sternal marrow revealed a cellular bone marrow with ample megakaryocytes and nondiagnostic changes consisting of only slight increase in plasma cells and slight granulocytic hyperplasia with occasional clusters of normoblasts. The basal metabolic rate was minus 12%. The venous pressure was normal. A urine culture was sterile. Biopsies of rectus muscle and skin revealed atrophy of epidermis and nondiagnostic, slight, perivascular, chronic inflammation.

On admission, the patient was provided a 200-mg. sodium diet. Generalized muscular weakness increased and she even noted easy fatigability of masticating and laryngeal

TABLE 3.—PLASMA LIPOPROTEIN STUDIES

		This Patient		
mg./100 cc.	*Normal*	*September, 1954*	*October, 1954*	*November, 1954*
Total cholesterol	230	477	320	427
Lipoproteins				
$-S_{1.21}>70$	22	24	7	35
40–70	29	66	38	152
25–40 (β_1)	180	1115	>212	940
20–25 (α_2)	13	71	19	35
1–10 (α_1)	228	188	75	143

Legend: Mean normal values and observed values in this patient. The normals stated for lipoproteins are means of values for women of 35 to 60 years of age. The $-S_{1.21}>70$, 40–70, and 25–40 fractions correspond, respectively, to S_f 20–100, 12–20, and 3–8, in determinations made at specific gravity 1.063.

TABLE 4.—OTHER LABORATORY PROCEDURES

		This Patient			
	Normal	*July 1954*	*September 1954*	*October 1954*	*November 1954*
Blood glucose, mg./100 cc.	60–110	107	80	—	—
Blood urea, mg./100 cc.	21– 42	25	21	36	27
Urea clearance, % of normal	75–140	165	75	—	—
Renal blood flow, female, cc./1.73 m.²/min.	1000	—	—	675	—
Renal plasma flow, female, cc./1.73 m.²/min.	600	—	—	324	—
Glomerular filtration rate, female, cc./1.73 m.²/min.	115	—	—	101	—
Filtration fraction (G.F.R./R.P.F.)	0.20	—	—	0.31	—
Plasma creatinine, mg./100 cc.	0.5–1.5	—	1.12	0.50	0.50
Serum uric acid, mg./100 cc.	3–6.5	2.9	3.5	—	—
Serum sodium, mEq./L	140–150	130	130	135	132
Serum potassium, mEq./L.	3.5–5	5.1	3.5	3.8	4.1
Plasma CO_2 mEq./L.	22–27	25	26	26	23
Plasma chlorides, mEq./L.	93–101	100	86	85	86
Serum calcium, mg./100 cc.	9– 11	—	9.7	8.7	9.1
Serum phosphorus, mg./100 cc.	2– 3.5	—	5.9	4.9	5.3
Congo red test, % residual at 1 hr.	40– 90	—	34 (amber urine)	41 (amber urine)	—
Bromsulphalein, % retained in 45 min.	neg.	7.5	13	—	—
Serum cryoglobulin	neg.	—	neg.	—	—
Phosphatase, alkaline, Bodansky units	2– 4		3.7	—	—
Cephalin-chol. flocculation	neg. in 48 hr.	1+	—	—	—

muscles. The intramuscular injection of 1.5 mg. of neostigmine methylsulfate was followed by no subjective or objective improvement. Intravenous infusions of calcium and of potassium did not alter the Q–T interval or increase skeletal muscular power. She gradually lost the ability to eat and to retain food; electrolyte and water balances were maintained by daily intravenous infusions. On the twenty-fifth day in the hospital, she developed paroxysmal auricular tachycardia, became dyspneic, and complained of a feeling of precordial oppression. Sinus rhythm was reestablished spontaneously after several hours. She was digitalized, but repeated attacks of tachycardia occurred, usually lasting 6 to 12 hours, which were unresponsive to carotid massage and prostigmine and which subsided spontaneously. On the twenty-sixth day in the hospital, the liver was palpable; marked postural hypotension and proteinuria were persistent and peripheral edema increased. On the thirty-first day, a profuse foul-smelling vaginal discharge was noted; multiple small vesicular lesions were found scattered over the mucosal lining of the vagina and 2 small ulcerated areas were present on the anterior surface of the cervix. A biopsy of the cervix showed extensive perivascular infiltration with amyloid.

Administration of 100 mg. of cortisone daily was begun on her thirty-eighth hospital day. She became progressively weaker and was unable to leave the bed. Nausea and vomiting were poorly controlled and she developed diffuse abdominal pain and tenderness; peripheral edema increased and bilateral pleural effusion developed. She became suddenly dyspneic, complained of intense precordial pain, and died on November 11, 1954, 46 days after initial admission.

A necropsy was performed by Dr. L. J. McCormack of the Department of Pathology. Microscopic examination revealed generalized primary systemic amyloidosis, evidenced wherever blood vessels or muscles were present. Sections of the lung showed abundant amyloid material in the alveolar wall and arteriolar walls, and surrounding the bronchi. All sections of the heart showed large amounts of amyloid deposition in endocardium, myocardium, and epicardium; sections of each valve revealed the diffuse presence of amyloid. Amyloid was present in the walls of coronary arteries and in the media and intima of the aorta. Splenic pulp was completely replaced by amyloid. There was extensive deposition of amyloid in the hepatic cords and portal spaces of the liver and in the walls of the pancreatic ducts and splenic vein. Sections of the kidney showed replacement of most of the glomeruli by masses of amyloid; amyloid was also seen in the walls of the arterioles and in the tubules. The adrenal contained deposits of amyloid in the zona reticularis and submucosal deposits were present in the bladder. There was extensive perivascular deposition of amyloid in the submucosal and muscle layers of esophagus, stomach, jejunum, and colon. The thyroid and skeletal muscles contained amyloid in vessel walls, and foci of amyloid were seen in the sinusoids of the bone marrow.

Additional pathologic diagnoses were: multiple pulmonary emboli of the small vessels of the lung, small pulmonary infarct of the right lower lobe of the lung, multiple recent submucosal hemorrhages of the gastrointestinal tract, and mild arteriosclerosis of the aorta.

Discussion. The patient was a middle-aged woman whose clinical course was characterized by severe hypotension, asthenia, muscular weakness, and the nephrotic syndrome with hypoproteinemia, proteinuria, edema, and lipemia. Necropsy disclosed widespread amyloidosis involving smooth, cardiac, and skeletal muscles, and affecting the blood vessels in practically every organ, especially in the parenchyma of the kidneys, the spleen, and the liver.

The case presented an unusually difficult diagnostic problem. The degrees of proteinuria, hematuria, and cylindruria were compatible with the nephrotic stage of chronic glomerulonephritis. Moreover, the electrophoretic pattern of the serum proteins with a decrease in albumin, elevations of $alpha_2$ and beta globulins, and low gamma globulin levels and, by ultracentrifugation, excessive concentrations of $beta_1$ and $alpha_2$ with normal $alpha_1$ lipoproteins, could not be differentiated from nephrotic glomerulonephritis according to the criteria of Lewis and Page[2]. However, careful examinations of the urinary sediment revealed no hyaline blood cell casts. In addition

instead of a reduction in glomerular filtration rate and a decrease in the fraction of plasma water filtered by the glomeruli, which is the usual pattern in the nephrotic syndrome of nephritis, the glomerular filtration rate was normal and the filtration fraction markedly elevated.

In adults manifesting the insidious onset of the nephrotic syndrome, intercapillary glomerulosclerosis (Kimmelstiel-Wilson's syndrome) must be excluded, but in our patient there was no hypertension, retinitis, or evidence of diabetes mellitus. The profound asthenia and hypotension were suggestive of Addison's disease, yet there was no typical pigmentation and adrenocortical function was normal by the ACTH test. The nephrotic syndrome and hypotension occasionally are manifestations of systemic lupus erythematosus; the urinary sediment of this patient was consistent with this diagnosis. However, the L. E. cell phenomenon could not be demonstrated in bone marrow or in peripheral blood.

Periarteritis nodosa has a variable and often bizarre clinical pattern and frequently is associated with glomerulonephritis; however, there was no history of allergy in this patient, no eosinophilia, no neuropathy, and a biopsy of skeletal muscle was nondiagnostic. Amyloidosis is not uncommon in multiple myeloma, and signs of myeloma may be masked by the amyloid disease. The total plasma proteins in myeloma are rarely, if ever, below 5.0 gm. per 100 cc. In this patient the total proteins were consistently less than 5.0 gm. per 100 cc.; Bence-Jones protein could not be found in the urine; azotemia was absent; and examination of the bone marrow showed no significant increase in plasma cells.

Primary systemic amyloidosis was believed to be the most likely diagnostic possibility in this patient, but positive proof was lacking. The Congo red tests were negative; they indicated retention in the blood of 34 and 41% of the dye one hour after injection; in each instance, because of proteinuria, dye (which is adsorbed to albumin) appeared in the urine. A renal biopsy was considered but was not performed. Recent experience[6] shows that this technique is of value in unraveling diagnostic dilemmas in renal disease. The appearance of lesions of the cervix uteri finally provided a biopsy site from which a positive diagnosis was established.

Many of the clinical features manifested by this patient can be explained by the extensive deposition of amyloid material in blood vessels, muscle, and parenchymatous tissue throughout the body. Pulmonary embolus was the immediate cause of death; the tendency to form clots may have been increased by the submucosal hemorrhage involving the entire gastrointestinal tract. Amyloid is believed to be formed from plasma protein possibly as precipitates of protein with mucopolysaccharide as a result of an immune reaction. Since amyloidosis has been thought to be related to collagen disease, and is a reversible process, at least in the early stages, steroidal therapy has been suggested as the only hope for an improvement[1]. Improvement after administration of ACTH and cortisone has been noted in 2 patients with primary amyloidosis involving the larynx[5]. On the other hand, Paulsen[7] reported that treatment with ACTH in 2 patients with generalized amyloidosis was followed by an increase in proteinuria and progression of their diseases. The intermittent administration of ACTH and cortisone for a total of 47 days in our patient did not alter the inexorable and progressive deterioration of her condition.

The elevated blood cholesterol parallels the reduced plasma proteins. Some years ago one of us (I.H.P.) suggested that this was due to the splitting of the lipoproteins in the kidneys with excretion of the protein in the urine and retention of cholesterol and other lipids in the blood. In the absence of better evidence, this explanation still seems valid.

The chronic hypotension with a marked orthostatic component is difficult to explain; extreme muscular weakness may have been a contributing factor, and there may have been some specific dietary deficiency. Extensive involvement of the heart and the blood vessels with amyloid also may have played an important part. The ACTH-eosinophil test and the excretion of 17-ketosteroids appeared to show adequate adrenal function. Hepatic function was only slightly impaired as measured by bromsulphalein retention (13%, compared with 0 to 10% normal).

Lindsay[3] published a complete analysis of the literature dealing especially with the cardiac effects of primary systemic amyloidosis. Cardiac failure was noted in 23 of 45 cases recorded up to 1946 and was a cause of death in 18. Our patient also showed some of the evidences of cardiac failure (nocturnal dyspnea, weakness, shortness of breath and edema), but it was somewhat obscured by the concurrent presence of a nephrosis-like syndrome. Lindsay does not mention the occurrence of chronic hypotension. Hypertension associated with primary amyloid had been recorded in only 4 cases according to him. Primary amyloidosis of the kidneys characterized by the nephrotic syndrome and hypertension has recently been reported[8] in one patient.

Summary. The course is described of a woman with primary amyloidosis in whom the diagnosis was proven by biopsy of the cervix uteri and necropsy. The unusual features were the nephrotic syndrome and persistent severe hypotension. Both phenomena may have been due to infiltration of the blood vessel walls and myocardium with amyloid.

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SUMMARIO IN INTERLINGUA

Syndrome Nephrotic e Hypotension Chronic Associate con Primari Amyloidosis Systemic. Reporto de un Caso

Es describite le caso de un femina con amyloidosis primari in qui le diagnose esseva confirmate per biopsia del cervice del utero e postea per constatationes necroptic. Le characteristicas inusual del caso esseva le syndrome nephrotic e un sever e persistente hypotension. Ambe iste phenomenos esseva possibilemente causate per le infiltration de amyloide a in le myocardio e le parietes del vasos sanguinee.

PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF

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JAUNDICE IN THE NEONATAL PERIOD

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THE compilation of material for a review is in essence a process of intellectual stock-taking. The subject of neonatal jaundice is, at first thought, clouded by confusion. Within the past few years, however, there has been such a dramatic increase in knowledge that the picture may now be described as complex rather than confused. Immediately prior to World War II very little was known of the etiology or pathogenesis of jaundice in the newborn except for obvious cases of extrahepatic bile duct atresia, congenital syphilis and septicemia. Practically all of the entities which will be discussed in this review were either not known or not clearly defined 15 years ago.

The period immediately after birth is an extremely critical time, and although the first day of life is the most crucial, the entire neonatal period is one of delicate adjustment. By convention the neonatal period is defined as the first 28 days of life and during this time jaundice is a sign of fickle significance. It may be relatively inconsequential or it may be of serious portent heralding a grave or even fatal disease. The jaundice may present as

a problem of relative simplicity in regard to both etiology and treatment, or as one of exceeding complexity.

Excessive breakdown of red cells, hepatocellular or parenchymal damage, and anatomical or organic biliary obstruction are the basic factors in the production of jaundice at any age. In infancy the operation of these causes of jaundice in such entities as erythroblastosis, sepsis, syphilis and congenital atresia of the bile ducts is fairly well understood. However, when all known causes of jaundice are accounted for, there still remains a large group with uncertain etiology. In the latter group are included cases which are still labelled physiological jaundice and also a number of ill-defined entities which are collectively designated as hepatitis.

The differential diagnosis of jaundice in the newborn may be difficult; a hemolytic jaundice is frequently complicated by obstructive elements and not infrequently the jaundice associated with the parenchymatous type of liver damage will appear clinically as a complete obstruction with pale stools and no urobilin in the urine. Many of the tests of liver function are of doubtful value at this age and their interpretation is even more difficult than in the adult. It is not surprising, therefore, that a biopsy may be the only possible way to establish a diagnosis. The pathologist in turn is confronted with a similar problem when he must determine whether the changes he finds are characteristic of a disease entity or merely the nonspecific reaction of the liver to injury.

"Physiological" Jaundice. The occurrence of jaundice in newborn babies in whom there is no evidence of disease has long been recognized. The degree of the jaundice is variable, but it is definitely more marked in premature infants[33]. The condition is usually regarded as innocuous, but the reports of Aidin, Corner and Tovey[2], and Claireaux, Cole and Lathe[9] demonstrate that severe "physiological" jaundice associated with prematurity may lead to bile staining of the basal nuclei of the brain. This occurrence of kernicterus indicates that "physiological" jaundice is not an entirely benign complication of the newborn period.

The old concept that icterus neonatorum merely resulted from the rapid hemolysis of red cells which normally occurs after birth received its first serious challenge when Mollison[42] demonstrated that the increased production of bilirubin during the first 10 days of life was only three times normal. Such a load would be rapidly cleared by a normal mature liver but in the newborn infant, particularly if the liver is immature, the pigment cannot be excreted as rapidly as it is produced. Hyperbilirubinemia results and the pigment is always of the indirectly reacting type.

The relation of birth weight to the degree of bilirubin retention has been investigated by Billing, Cole and Lathe[6]. They found that some infants who were mature by weight showed plasma bilirubin levels as high as 7 mg./100 cc. but that concentrations of the pigment as a rule were higher in small premature infants, in whom levels over 12 mg./100 cc. were often seen. The type of bile pigment present in these cases was determined by the chromatographic method described by Cole and Lathe[10] and 90 to 95% was found to be indirectly reacting pigment. These authors have also compared the excretory function of the liver of the premature newborn infant with that of the normal adult. They calculated that the liver of the premature infant can excrete bilirubin at the rate of only 1 or 2% of the normal adult liver. This work supports the concept that "physiological" jaundice is due to

a reduced capacity of the immature liver to excrete bilirubin.

The exact mechanism for the excretion of bilirubin is not known. Malloy and Lowenstein[37] suggested that the transformation of indirectly reacting bilirubin to directly reacting pigment was an essential stage in the process and it is possible that the high levels of indirectly reacting bilirubin in the plasma of these cases may be due to a failure of the liver to transform the pigment.

A different approach to the problem of "physiological" jaundice has been made by Emery[18,19], who considered that some cases might be explained by a general impairment of liver functions consequent on the physiological involution of the left lobe of the liver which occurs at birth. During fetal life the left lobe of the liver possesses a triple blood supply. In addition to the hepatic artery and portal vein supply it receives branches from the umbilical vein. The blood flowing through the right lobe of the liver is not enriched by this "arterial" element. The sudden change in the circulation which occurs at birth deprives the left liver of its rich oxygenated blood supply. The whole liver decreases in size after birth but the change is more pronounced in the left lobe. The liver of the newborn child at necropsy sometimes shows a striking difference between the left and right lobes. The left lobe is much darker in color and microscopic examination of this lobe shows shrunken liver cell cords and dilated sinusoids. These changes in the liver are thought by Emery to be an accentuation of the normal physiological involution of the liver. However, he has not as yet correlated these anatomical observations with the occurrence of hyperbilirubinemia and jaundice in the newborn. One cannot deny the possibility that this mechanism may play a part in some cases of neonatal jaundice, but it is difficult to imagine how a disturbance of the liver blood supply could produce the specific defect of bile pigment excretion postulated by Billing *et al.*[6].

It is safe to conclude, however, that in the immediate postnatal period the liver is presented with an increased load of bilirubin due to the rapid breakdown of red blood cells. The liver is often unable to clear the plasma of the excess bilirubin and this defective function is directly related to the degree of immaturity of the infant. The jaundice is often mild and of no significance, but the term "physiological" jaundice is dangerously misleading, because in the small premature infant, high levels of indirectly reacting bilirubin may be associated with the production of pigmentation of the brain and the death of the infant.

Erythroblastosis Fetalis. The primary cause of jaundice in erythroblastosis fetalis, or icterus gravis neonatorum, is the greatly increased hemolysis of damaged red cells. Crowding and displacement of the liver cells with foci of hematopoiesis may play a secondary role. These infants are not jaundiced at birth, probably because the maternal liver is able to excrete any excess fetal bilirubin. Icterus is usually noticed within the first few hours of life.

This hemolytic jaundice is, however, frequently augmented by an obstructive component; the stools become pale, bile appears in the urine and the concentration of directly reacting pigment increases in the plasma. The obstruction may become complete and last for several months[28]. There is little doubt that some of these cases have been diagnosed as congenital biliary atresia. Hsia, Patterson, Allen, Diamond and Gellis[32] found in their series of 156 cases of prolonged obstructive

jaundice in infancy that 23 (15%) were associated with erythroblastosis and that 10 of these had been admitted for surgery with a diagnosis of biliary atresia. Similar figures were obtained in a series of cases of obstructive jaundice in the newborn at The Hospital for Sick Children, Toronto.

The cause for the obstructive component in the jaundice of erythroblastosis is not completely understood. In cases that come to postmortem examination, plugging of small bile canaliculi and cholangioles is commonly seen. This plugging or inspissation of bile is related to the degree of jaundice but does not seem to be dependent upon either the amount of extramedullary blood formation or parenchymal damage.

Extramedullary blood formation, particularly in the liver, is a characteristic feature of erythroblastosis. It is, however, an extremely variable finding and cannot be completely correlated with the apparent degree of severity of the disease. Erythroblastosis due to ABO incompatibility may be accompanied by deep jaundice, but in our experience with two fatal cases there was very little evidence of extramedullary blood formation.

Early reports suggested that damage to the liver cells is frequently seen in fatal cases of erythroblastosis[24,30,59]. The changes described include swelling, fatty infiltration and necrosis. In our opinion these changes may be the nonspecific results of anemia and anoxia. Craig[11], however, considered that the mid-zonal hepatic necrosis which he frequently saw in fatal cases of icterus gravis neonatorum was not the type of necrosis usually seen in anoxic states.

A suggestion has been made by Polani[47] that the liver cells may be damaged by the direct effect of an antibody. He described necrotic lesions occurring in the livers of albino rats made anemic with hemolytic serum. With recovery, prompt regeneration of the liver parenchyma occurred without residual fibrosis. Hepatic necrosis did not occur in control rats either exposed to low oxygen tensions or rendered anemic by the injection of phenylhydrazine. He concluded that the liver cells were damaged by the direct action of the antibody, and suggested that a similar mechanism might be operative in erythroblastosis fetalis. Cases of erythroblastosis that are due to one or more of the Rh-Hr factors are the most common. In these the antigen is contained only in the red cells of the fetus and there is no evidence that any maternal antibodies are formed other than those which are stimulated by the fetal red cell antigens. Hence, it is difficult to postulate the action of an anti-liver antibody in the human.

Although the obstructive jaundice of erythroblastosis may be severe and last for as long as 4 months, it does, invariably in our experience, clear, leaving no evidence of hepatic dysfunction. The formation of pigment stones is a rare complication; Lightwood and Bodian[35] described two cases in which such stones obstructed the main bile ducts.

There have been a few reports of permanent hepatic damage following icterus gravis. Hawksley and Lightwood[30] reported 19 cases of erythroblastosis, and 7 of these showed hepatic fibrosis which varied from slight intercellular fibrosis to well marked cirrhosis. Zuelzer and Bigler[59] recorded one similar case. However, these reports were written before the etiology of erythroblastosis was known, and the diagnostic criteria were not specific; hence no valid conclusions can be drawn from these early records. Craig[11] reported several erythroblastotic in-

fants who died with "infantile cirrhosis" and claimed that erythroblastosis was a cause of cirrhosis in a small percentage of cases. Two of these cases, however, were later presented by the same author and an associate[12] as examples of infantile giant-cell hepatitis, a condition which at this later date they considered to be of viral origin. It is possible that the three cases of "infantile cirrhosis" described by Lightwood and Bodian[35] fall into the same category.

A majority of the reports of the late effects of erythroblastosis on the liver have been based on necropsy material, but as most of the cases where there is evidence of prolonged obstructive jaundice do not die, little is actually known about the state of liver in this condition. Harris, Andersen and Day[28], performed liver biopsies on a few such cases and reported that the liver showed many multinucleated giant cells several months after the onset of the jaundice. This picture is, however, in all essential respects, similar to that described in cases of infantile hepatitis by Craig and Landing[12]. Multinucleated liver cells are probably not peculiar to any one disease but rather represent a nonspecific reaction to injury.

Skelton and Tovey[53] described two probable cases of erythroblastosis with extrahepatic biliary atresia and suggested that the atresia was due to the fibrosis of the plugged bile ducts. McGeehan, Butchart and Walker[39] discussed this theory and concluded that as only five cases have been reported in which erythroblastosis and biliary atresia had been found to co-exist, an etiological relationship between the two conditions had not been established.

The clinical and pathological evidence indicates that obstructive jaundice is a common complication of hemolytic disease of the newborn. The cause of the obstructive component is not known. Although the jaundice may be prolonged, it almost invariably resolves and permanent damage to the liver is extremely rare. Apparent sequelae may be due to coincidental diseases such as viral hepatitis or congenital atresia of the bile ducts.

Hepatitis. Until recently it has been believed that viral hepatitis does not occur in infants under the age of 2 years. One of the reasons for this widespread belief is the fact that clinically recognizable infectious hepatitis (hepatitis virus A) is a rare complication of pregnancy. DeLee and Greenhill[15] reported the combination in only 10 of 72,000 pregnant women and similar figures were given by Mickal[41] who found that the infants showed no effects of the maternal disease and that there was little tendency for maternal hepatitis to lead to premature birth.

On the other hand, Stokes *et al.*[54] have estimated that 0.2 to 0.5% of the general population carry the virus of serum hepatitis (hepatitis virus B) in their blood. These authors reported the detection of this virus in 3 blood donors and estimated, on the basis of the people these donors had infected, that it had been present in the blood of the donors for at least 5½ years, 3 years, and one year respectively. The carriers gave no history of jaundice and had no symptoms.

Stokes and his associates[55] have also suggested that transplacental transmission of serum hepatitis from an apparently healthy mother to the fetus may occur. They reported an infant who developed jaundice at the age of 2 months. Infection through the birth canal was ruled out because the baby was born by Cesarean section and transmission by means of breast milk could be eliminated since the baby was not breast fed. The sera of mother

and infant produced hepatitis when injected into human volunteers. The mother gave no history of liver disease. This case is important because it was one of a group of 12 infants in whom jaundice began shortly after birth. All these infants showed the same changes in the liver at necropsy. The changes were those which have been described by other investigators as infantile hepatitis or giant cell hepatitis[12,16,28,52].

We have seen 8 cases of giant-cell hepatitis and our findings are similar to those reported by the above. The liver shows diffuse disorganization of intralobular architecture, although the lobular pattern is usually discernible. The liver cell cords are broken and replaced by irregular masses of bizarre multinucleated giant cells which often resemble osteoclasts. Large islands of hemopoietic cells may be seen in close relationship to the giant cells. The giant cells may contain heavy deposits of stainable iron. This feature was stressed by Dible *et al.*[16] who also pointed out that the histological changes were essentially those of diffuse liver cell necrosis with the sequelae of histiocytic activity, fibrosis and bile duct proliferation. They thought that the multinucleated giant cells were the result of attempts at regeneration of isolated cells which in the infant may give rise to this unusual picture. Little is known about the course of nonfatal giant-cell hepatitis, but Craig and Landing[12] reported 2 cases which were diagnosed by liver biopsy in early infancy and later developed hepatic cirrhosis. Both these children died in their third year of life, and necropsy on one showed an advanced interlobular cirrhosis with large irregular nodules of regeneration. No trace of active hepatitis and no large multinucleated cells were observed.

The etiology of infantile giant-cell hepatitis is not known, and it is not even certain that the condition represents a single disease entity. It is possible that some cases of neonatal giant-cell hepatitis may be due, as suggested by Stokes[55], to a transplacental infection of the fetus with hepatitis virus B. There has been, to our knowledge, no proven instance of intrauterine infection with hepatitis virus A. Postnatal infection may of course occur; Zuelzer[58] reported an epidemic of diarrhea in a premature baby unit, 50% of the infants became jaundiced and 50% of the jaundiced infants died. The livers of the fatal cases showed the picture of giant-cell hepatitis. It was considered on clinical and epidemiological grounds that the epidemic was probably caused by hepatitis virus A.

We are probably justified in concluding that a giant-cell hepatitis may be produced by infection, which may be transplacental, with the hepatitis viruses A or B. There is, however, no justification for the assumption that the term giant-cell hepatitis is synonymous with infectious or serum hepatitis. The multinucleated giant cells are probably the result of unruly liver cell regeneration and it cannot be said that liver cell regeneration is specific to one disease any more than one can claim that liver cell necrosis is peculiar to one type of liver injury. Multinucleated giant cells may be seen in cases of biliary atresia although in this condition, they are usually few in number and show obvious degenerative changes.

The familial incidence of giant-cell hepatitis is of interest. Two of Dible's cases[16] were successive siblings and the third child of the mother was stillborn and macerated. No serological evidence of hemolytic disease was found so that the possibility of erythroblastosis fetalis was excluded. A familial occurrence was also reported by Scott, Wilkins and Kessler[52] and in this

family 3 infants died with giant-cell hepatitis and the mother and 2 surviving children showed abnormal cephalin flocculation tests. The 2 surviving children, who were symptomless, were born between the first and second jaundiced infants. The authors considered that the mother was a carrier of the virus of serum hepatitis. This is an attractive theory but it is possible that some genetically determined inborn error of metabolism, similar to that which produces the parenchymal liver cell damage in galactosemia, is operative in these familial cases.

We must conclude, therefore, that although giant-cell hepatitis is a definite morphological entity it is not certain whether it represents a single disease process. The development of safe liver biopsy techniques for use in infants may provide additional information about this condition. We may find that the condition is not as rare as previously thought and that progression of the disease may be an important cause of childhood cirrhosis. The question of the viral etiology of some or all of these cases must await the day when methods for the identification of the hepatitis viruses become available.

HEPATITIS DUE TO KNOWN VIRUSES. In addition to the hepatotropic viruses of infectious and serum hepatitis, which may possibly be a cause of neonatal jaundice, other viruses can cause extensive liver damage at this age period. In these cases widely disseminated lesions are usually present but the viruses often show a peculiar predilection for the liver and the damage is most extensive in this organ. Hass[29] reported a fatal disease in a prematurely born infant. The condition was characterized by hepato-adrenal necrosis and the presence of intranuclear inclusion bodies in the cells of the liver and adrenal cortex. He assumed that the lesions must have been produced by a filtrable virus. The virus was, almost certainly, herpes simplex, and since that time there have been several proven instances of the hematogenous spread of this virus causing hepatitis in the newborn and in older infants[22,48,49,60]. The lesions in the liver often resemble abscesses and may easily be dismissed as the result of the secondary bacterial invasion which may develop in disseminated herpes infection. Pugh *et al.*[48] have emphasized this point and have stressed the importance of examining direct smears of the lesions at necropsy and of saving serum and infected tissues for egg and animal inoculation. Microscopic examination shows that the "abscesses" of the liver consist of focal areas of necrosis with a peripheral rather scanty infiltration with inflammatory cells. Many of the parenchymal cells show typical acidophilic intranuclear type A inclusions. This is apparently not a congenital infection but one acquired either during birth or immediately after birth. Not infrequently the mother is found to have an herpetic lesion.

Another rare cause of neonatal jaundice is cytomegalic inclusion disease. The infection is acquired *in utero* from a mother with no clinical evidence of disease. The diagnosis of the condition has almost always been made after death by the finding of the typical intranuclear and cytoplasmic inclusions in the cells of the affected organs. This must be an ubiquitous infection, as identical inclusions are frequent incidental findings in the salivary glands of infants dying of various diseases[20]. Wyatt, Saxton, Lee and Pinkerton[57] have pointed out, however, that the congenital disease associated with visceral dissemination of the infecting agent is usually fatal. Jaundice in the newborn is a common symptom of the disseminated disease, and the presence

of hepatomegaly, splenomegaly and many nucleated red blood cells in the peripheral blood accounts for the frequent misdiagnosis of the condition as hemolytic disease of the newborn due to a blood group incompatibility. The Coombs test is negative. Pathological evidence for the occurence of hepatitis with progression to cirrhosis has been presented by Wyatt *et al.*[57] and they consider that the disease is caused by a specific virus. The virus has not yet been isolated although numerous attempts have been made. However, a similar disease in the salivary glands of rodents has almost certainly been proven to be due to a virus[51]. The recent report by Margileth[38] of the diagnosis of the disease during life offers some hope that more extensive viral studies may be possible. The diagnosis was made by the demonstration of the inclusions in unstained epithelial cells from centrifuged urine. The cells may be stained with hematoxylin and eosin.

BACTERIAL AND PROTOZOAN INFECTIONS AND THE LIVER. Coincident with the increase in knowledge concerning the etiology and pathogenesis of neonatal jaundice there has been an almost complete disappearance of the conditions which were once the most important causes of pathological jaundice at this age. Icterus associated with congenital syphilis or septicemia is now uncommon. Only 2 cases of congenital syphilis were seen in the neonatal period at The Hospital for Sick Children in 1953 and one of these was admitted with the diagnosis of erythroblastosis fetalis. This compared with 6 cases in 1939. It is interesting to note that whereas Robert Hutchison in his "Lectures on Diseases of Children"[34] considered that congenital syphilis was a "not uncommon cause of jaundice in infancy," Nelson[45] does not even list syphilis amongst the causes of neonatal jaundice. Severe icterus usually accompanies neonatal septicemia and the coliform organisms are the most important pathogens at this age. In our experience the jaundice is due to hepatocellular damage and not to hemolysis.

Infection with the Toxoplasma protozoan may occur *in utero*[21], and although congenital toxoplasmosis is a rare cause of neonatal jaundice, more and more cases are being recognized. A meningoencephalitis is usually the most prominent feature of the condition and the active disease may be diagnosed by demonstrating the parasite in smears of the cerebrospinal fluid and by growing the organisms in mouse peritoneum or in tissue culture. The complement fixation test and the dye test will be positive in the mother.

Inborn Errors of Metabolism. GALACTOSEMIA. Hepatocellular damage is apparently an essential feature of galactosemia. This disease is a congenital familial defect of metabolism characterized by an inability to metabolize lactose and galactose in the normal manner. The essential features are jaundice, severe malnutrition, hepatomegaly, and the presence of galactose in the urine. Galactose causes reduction of Benedict's solution, but it may be distinguished from glucose by differential fermentation with yeast or by paper chromatography. The occurrence of jaundice which usually commences on the third day, is almost constant. The livers of the fatal cases show varying degrees of cellular degeneration. The most severe cases exhibit complete disruption of lobular architecture and extensive loss of parenchymal cells with collapse and condensation of the reticulin framework. The cells may be arranged in short columns or they may form distinct tubular structures. The cholangioles are usually plugged with bile. The final picture is one of diffuse hepatic fibrosis which is often pericel-

lular and not unlike diffuse syphilitic cirrhosis. Many of these cases are misdiagnosed both by the clinician and the pathologist. A recent review at The Hospital for Sick Children, Toronto, of 46 cases of hepatic cirrhosis who died under the age of 6 months showed that in 9 the diagnosis of galactosemia was probable[3]. These cases had been labeled as idiopathic cirrhosis or intrahepatic biliary atresia. The histological findings in this disease are not specific, but we think that a presumptive diagnosis of galactosemia can be made from a microscopic examination of the liver.

The metabolic defect is generally thought to be one which affects the enzyme systems concerned with conversion of galactose to glucose, but the mode of production of the hepatic damage is not known. Bell, Blair, Lindsay and Watson[4,5] thought that the failure to metabolize galactose resulted in a fatty infiltration of the liver which in turn led to the diffuse cirrhosis. This may be a factor in some cases but the fat content of the liver in 2 proven cases we have seen at necrospy was well within the normal range. Bruck and Rapoport[8] proposed a theory that the pathological manifestations were due to a direct toxic effect of high levels of galactose.

The importance of recognizing the condition as soon as possible after birth cannot be overemphasized, as the simple expedient of replacing the milk in the diet by a milk substitute halts the entire process and enables the infant to develop normally.

CONSTITUTIONAL HYPERBILIRUBINEMIA. Impairment of the excretory functions of the liver which may manifest itself in the neonatal period is not limited to the immature infant. Crigler and Najjar[13] have described 7 cases of congenital familial nonhemolytic jaundice. In these infants, the abnormality appeared to be inherited as a recessive characteristic. There was elevation of the indirectly reactive serum bilirubin. The prognosis was poor, and 5 of the infants died within the first year of life with kernicterus. At postmortem examination, the liver showed bile thrombi in the small bile passages. This condition may possibly be related to familial nonhemolytic jaundice which is usually not diagnosed until early adult life[14].

A new disease which falls into this group of constitutional hyperbilirubinemias was described by Dubin and Johnson[17]. It differs from the familial nonhemolytic jaundices in that there is intermittent bilirubinuria and elevation of directly reacting pigment in the serum. The liver is grossly and histologically colored by brown pigment in the parenchymal cells. This disease is rare and it has not yet been described in infancy, although one of the 12 cases described by Dubin and Johnson was said to have been jaundiced from birth.

Congenital Atresia of the Bile Ducts. Congenital anomalies of the biliary tree, although important causes of jaundice in infancy, are in fact uncommon conditions. Ahrens, Harris and MacMahon[1] were able to find only 250 cases of extrahepatic biliary atresia in the literature, and Moore[43] estimated that the malformation occurred once in every 27,000 births. The familial incidence of the condition has been investigated by Whitten and Adie[56] who reported the occurrence in 2 siblings. They were able to find only 4 previous instances and 4 suggestive instances of the familial occurrence of congenital biliary atresia.

There has been little advance in our knowledge of the condition since the classical description by Holmes in 1916[31]. He suggested that surgical treatment was possible in some cases and stated that in 16% of the recorded

cases the anatomical relations were such that operative relief was theoretically possible. Gross[26] reached similar conclusions and claimed that 27 (18%) of 146 babies operated upon "had a condition which presumably could be remedied by an anastomotic procedure." Of these 27 cases, 12 were relieved of their jaundice and 15 had died. Thus of 146 cases, 12 could be claimed as cures (8%). Even this low figure would seem to be a little optimistic. Gray, Dushane and Heneger[25] in a review of the literature were able to find only 15 cases of biliary atresia which had been successfully treated by anastomosis. Redo[50] reported that 10 of 27 cases (37%) had conditions amenable to surgery but in only 2 were really successful results achieved. He concluded that the prognosis was poor and estimated the average span of life of patients with anomalies not amenable to surgery as 10 months.

Intrahepatic atresia of the biliary tract is an anomaly which seems to be compatible with much longer life than extrahepatic biliary atresia. Although the interlobular ducts may be absent or few in number in extrahepatic atresia, cases of pure intrahepatic atresia may occur with an intact and patent extrahepatic biliary system. This condition is, however, rare and to date only 16 cases have been reported[1,36,44]. The essential pathology of the condition was described in detail by MacMahon and Thannhauser[36] who designated the anomaly as congenital dysplasia of the bile ducts or acholangic biliary cirrhosis. The absence of the interlobular bile ducts in their case was accompanied by marked proliferation of periportal cholangioles and all transitions from mature liver cells to cholangiolar epithelium could be followed. They cited these features as evidence to favor the hypothesis of Hammar[27] that the liver cells arise from a liver cell anlage, that cholangiolar epithelium is derived from liver cells and that the interlobular ducts are derived from a separate bile duct anlage.

The obstruction to the flow of bile in these cases is incomplete, (unless accompanied by extrahepatic atresia), parenchymal cell damage is minimal and portal fibrosis slight. The condition has certain features in common with the so-called primary biliary cirrhosis of adults, the serum lipids are elevated and, if the child lives long enough, xanthomatosis may develop. We have had the opportunity recently to study a case of intrahepatic biliary atresia in a 3-month-old infant and the morphological and biochemical findings were similar to those described by MacMahon and Thannhauser[36].

The etiology of the various anomalies of the biliary tree remains a mystery but the recent report by Pappenheimer, Cheever and Salk[46] of the natural occurrence of both intrahepatic and extrahepatic biliary atresia in mice may provide a method for the investigation of these conditions.

The Diagnosis of Liver Disease. The difficulties of diagnosis of liver disease in children are even greater than in adults. The problems have been emphasized by Harris, Andersen and Day[28] who investigated 50 infants with persistent signs of biliary obstruction due to causes other than bile duct atresia. Clinically these patients all had jaundice, pale stools and dark urine and the laboratory studies were similar in all. They found that they were sometimes able to differentiate these cases from biliary atresia but in others surgical exploration of the biliary tract with liver biopsy and cholangiography was necessary. This has been true in our experience also, and we think that liver biopsy is often essential for the diagnosis of the var-

ious conditions. We would like to emphasize, however, that the pathologist may often be able to do no more than describe the morphological changes, because a particular histological pattern may be common to several diseases. With this obvious limitation in mind, the clinician has to weigh carefully the advantages which may accrue from examination of a liver biopsy against the risks of such a procedure. In most North American centers liver biopsies are taken by an open abdominal operation. This is understandable, and often essential in order that the biliary tree may be explored. There are few reports in the literature of the use of the needle biopsy technique in children. Gillman and Gillman[23] reported 181 needle biopsies without mishap in South African Bantu children with pellagra. Meneghello, Espinoza and Coronel[40] reported 89 needle biopsies on 29 children without accident and with only 3 failures to obtain liver tissue. Bras[7] has done 400 biopsies in Jamaican children with no fatalities and only one significant complication. There is no doubt that needle biopsy would be an extremely useful adjunct to the diagnosis of liver disease in children and that it would yield information not obtainable by any other means except an open operation. The information available would seem to suggest that under carefully controlled conditions needle biopsy of the liver in infants and children need not be attended by any greater risk than in adults.

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PREVENTIVE MEDICINE AND EPIDEMIOLOGY

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THE IMPACT OF TUBERCULOSIS ON HUMAN POPULATIONS

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THE essential facts in the natural history of tuberculosis have been known for some 75 years. During that time the reported mortality from the disease in the United States has decreased about 95%, yet little progress has been made in identifying and controlling the specific factors responsible for that change. There is a temptation for sanatoria and official and voluntary health agencies to take credit for the decline which has occurred, but with little factual evidence upon which to base such claims. Meanwhile, until the actual causes can be determined there is no assurance that the disease can ultimately be controlled, in the sense that available measures will eliminate effectively the increased frequency that still occurs with changed environment. Eradication is even more problematical.

MULTIFACTORIAL CAUSATION. Winslow[66] has aptly pointed out that the tubercle bacillus is not *the* cause of tuberculosis, but *one* of the causes. There is perhaps no better example of a disease of multifactorial causation than tuberculosis. For many years after the discovery of the tubercle bacillus it was generally assumed that the level of tuberculosis in a given population depended solely upon the distribution of the organism among the people. Cut off the sources of infection and the disease would disappear. Families were considered the major factor in transmission and ordinances were directed at spitting and dust control.

Epidemiological studies progressively made evident that the disease tuberculosis depends upon many factors besides simple contact between parasite and host. Fifty years ago the

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tuberculin test demonstrated that in European cities infection with tuberculosis was practically universal, yet only some 10% of people developed clinical tuberculosis. Why was infection not synonymous with disease? Why was tuberculosis a disease of infants and young adults, while old people and children from 5 to 10 appeared to be less susceptible? Knowledge of the causative organism did not answer all of these riddles.

The importance of environmental factors upon morbidity and mortality of tuberculosis has long been recognized. In a disease where the invasive powers of the agent are in such delicate balance with resistance of the host, factors which substantially augment the virulence of the causative organism or depress the resistance of the host logically will determine the trends of the disease. There is much evidence that general improvement in standards of living in our so-called western civilization during the past 100 years has played an important role in the phenomenal decline in tuberculosis. More adequate nutrition, reduction in overcrowding, smaller families, shorter hours of labor and less heavy physical work have done much to lessen and lighten the physical stress and strain of living. How such ecological factors operate and their relative importance in the course of tuberculosis in a human population are still major problems facing the epidemiologist.

THE TIME FACTOR. Basic difficulties in dealing with the epidemiology of tuberculosis are: the slow growth of the organism, the generally indolent reaction of the host, and the chronic, recurrent type of disease. It was primarily the time factor which so long concealed the infectious nature of tuberculosis. With intervals of a year or more between the development of successive cases in a family, heredity seemed a more plausible theory of causation than infection. Even with the causative agent known, the long and variable incubation period, the insidious onset and long duration of the disease have complicated seriously the tracing of sources of infection, recognition of modes of transmission, and evaluation of treatment and prognosis. This slow evolution and chronic course of tuberculosis calls for epidemiological treatment different from that to which we are accustomed in the study of acute communicable diseases. With an incubation period that is variable and indefinite, a course that may vary from months to many years and a fatality that can be computed finally only decades after onset, our ordinary methods of attack rate, prevalence and case fatality have little meaning. The time factor becomes an essential feature in evaluating the impact of tuberculosis on a population. Records must be kept in a form that makes possible valid comparison of the behavior of the disease in succeeding intervals of time as well as providing a measure of end results.

THE BIOLOGICAL BALANCE BETWEEN HOST AND PARASITE. Unlike the agents of most acute infections the tubercle bacillus is in relatively stable biological equilibrium with its human host. At times the parasite and again the host seems to be in the ascendency. In a virgin population with conditions favorable for transmission, as among the Eskimos, tuberculosis may become an epidemic disease, having a rapid course and high fatality. In North America and western Europe for the past half century, barring wars and other major catastrophies, the biological balance has been in favor of the human host. Rates of infection, morbidity and mortality have fallen steadily until ultimate control now seems a possibility. In

many parts of the world, however, tuberculosis still remains the leading cause of death and disability, with no assurance that the balance will be substantially altered in the near future.

The implications of the biological balance are such as to require brief consideration of the natural history of tuberculosis in man. Infection may take place any time in life after birth. During childhood it tends to occur at a steady rate, as indicated by the tuberculin reaction. In young adult life the curve begins to level off, with minor increments during the later years. In tuberculosis, however, infection is not synonymous with disease. While 50% of the adult population may at some time have been infected, probably only 3 to 5% in this country ever develop active disease. What, then, are the factors which determine whether infection of the individual results in progressive tuberculosis or an inactive focus without clinical significance? What are the determinants of recovery or death in the patient with active disease?

Since susceptibility to infection with the tubercle bacillus seems to be moderately high and relatively constant in different races and in various parts of the world, the most important single variable in determining progressive disease would seem to be host resistance[54]. Such an hypothesis is consistent with the well known variations in morbidity and mortality according to age, sex and race. That such native or inherent resistance is subject to wide fluctuations in the individual from time to time is well recognized. It is also clear that native resistance may be inadequate to protect the individual against such unfavorable environmental factors as severe physical stress and malnutrition, so convincingly demonstrated in recent wars.

The identification and evaluation of environmental factors in the etiology of tuberculosis will be considered later. Suffice it to point out here that the development of tuberculosis in man is a biological struggle between the invasive powers of the agent and the resistance of the host, modified by a variety of environmental factors. In this country for nearly a century steadily declining mortality and morbidity, and more recently infection rates also, indicate that the balance for many decades has been in favor of the human host[32a]. Recent events in Europe rudely remind that this favorable trend is reversible. Equally sobering is the thought that in a number of the larger areas of the world tuberculosis is still the leading cause of death.

Host Parasite Variations. THE INFECTIOUS AGENT. While man is susceptible to infection with the bovine and possibly the avian types of tubercle bacilli, the great bulk of tuberculosis in man is due to the human type of bacillus. In 1928, Möllers[48] estimated that 90% of all tuberculosis in man was due to the human type of bacillus. On the basis of the relatively rapid decline of extrapulmonary tuberculosis the proportion of bovine infection in man should be substantially less today. Since accreditation of dairy herds and the almost universal adoption of pasteurization in the United States, human infection with bovine tubercle bacillus has become of minor importance.

In Great Britain, Francis[31] estimated in 1950 that approximately 20% of all cattle and 40% of dairy cattle were infected. He also points out that while the majority of tuberculous infections in man remain inactive, in cattle nearly all infections result in slowly progressive bronchopneumonia from which tubercle bacilli are excreted. In rural areas especially, pasteurization of milk is far from complete.

The relative frequency with which the bovine bacillus occurs in human patients in Denmark according to age and type of tubercle bacillus is illustrated by Holm (After Sigrid Holm) in Table 1[39]. While a larger proportion of children showed pulmonary lesions and excreted tubercle bacilli soon after conversion of the tuberculin reaction, the number of adults who developed progressive tuberculosis after several years was much higher. In the same article, Holm also showed that the tuberculin reaction rates in children were low in those parts of the country where cattle were free from tuberculosis and much higher in areas where the disease still persisted.

TABLE 1.—RELATIVE FREQUENCIES OF BOVINE TYPE BACILLUS IN PATIENTS WITH PULMONARY AND EXTRAPULMONARY FORMS OF TUBERCULOSIS BY AGE GROUPS; DATA COMPILED FOR PATIENTS LIVING ON JANUARY 1, 1944, DENMARK

	Pulmonary Tuberculosis			*Extrapulmonary Tuberculosis*		
	Total Human and	*Bovine*		*Total Human and*	*Bovine*	
Age Groups	*Bovine*	*Number*	*Per Cent*	*Bovine*	*Number*	*Per Cent*
Total	11,072	384	3.5	1,454	204	14.0
0–4 years	473	18	3.8	91	31	34.1
5–9 years	476	15	3.2	71	20	28.2
10–14 years	519	7	1.3	43	7	16.3
15–19 years	1,457	51	3.5	130	23	17.7
20–24 years	2,282	68	3.0	190	24	12.6
25–29 years	1,787	67	3.7	177	29	16.4
30–34 years	1,267	42	3.3	130	18	13.8
35–39 years	860	30	3.5	115	13	11.3
40–44 years	563	23	4.1	105	10	9.5
45–49 years	413	13	3.1	81	7	8.6
50–54 years	295	15	5.1	73	5	6.8
55–59 years	242	12	5.0	63	9	14.3
60–64 years	171	9	5.3	61	5	8.2
65–69 years	120	5	4.1	43	2	4.7
70 and over	139	9	6.5	70	1	1.4
Unknown	8			11		

Source: Holm, J.[39]

The avian tubercle bacillus, in addition to being the cause of serious losses in the poultry industry, gives rise to tuberculosis in certain domestic animals. Swine and cattle are most frequently infected and the lesions tend to be limited to the lymph nodes. The disease is seldom widespread or destructive. Human infection with avian tubercle has been reported from time to time but confirmed cases have been extremely rare. In fact it is seriously questioned[54] whether any reported case of avian tuberculosis in man has been satisfactorily confirmed.

Any evaluation of the factors involved in the biological balance of a disease must include the virulence of the causative organism as well as the resistance of the host. In the case of smallpox it seems clear that the striking decline in fatality during the past 50 years is due not only to modification of the disease by vaccination but also to an actual mutation of the virus in many parts of the world. As a result the smallpox which does occur in this country is a mild disease, while the malignant smallpox which is occasionally introduced carries a fatality of 15 to 50%.

In tuberculosis the relative stability of the causative organism is one of the characteristics of the disease. To be

sure, attenuation of virulence for laboratory animals is known to occur under cultivation *in vitro,* as the Saranac R-1 strain and the bovine strain of Calmette and Guerin which became the basis of BCG. Occasionally tubercle bacilli of less than average virulence have been isolated from human lesions, but in general freshly isolated strains have shown remarkably uniform virulence for laboratory animals. Larmola[44] has reported wide variations in form, stainability and pathogenicity of bacilli isolated from human lesions. He states that it is difficult to draw the line between pathogenic and nonpathogenic forms but concludes that atypical strains are not common in typical clinical cases of tuberculosis.

TABLE 2.—TUBERCULOSIS MORBIDITY RATES IN TWIN INDEX FAMILIES

General	*Husbands*	*Parents*	*Siblings*	*Dizygotic Twins*	*Monozygotic Twins*
1.37	7.1	16.9	25.5	25.6	87.3

Source: Kallmann, F. J., and Reisner, D.[42]

THE HUMAN HOST. Wide variations in the reaction of certain population groups to first introduction of tuberculosis early pointed to the importance of the host factor as a determinant in morbidity and fatality of the disease. Familiar examples are the Senegalese troops brought to France in World War I, the American Indians when uprooted from their native habitat and herded on reservations. In both cases the reaction to infection was acute, the course of the disease epidemic and the fatality appalling. Pathologically the lesions tended to be generalized, with little evidence of systemic resistance.

Genetic resistance of laboratory animals to tuberculosis was clearly demonstrated by Wright and Lewis[68] at the Henry Phipps Institute in 1921. By inbreeding guinea pigs they were able to establish families with marked differences in resistance to tuberculosis. Their work has been amply confirmed by Lurie[46] and others.

In man, innate resistance to tuberculosis is much more difficult to demonstrate. In different races there seem to be wide variations in native resistance. It has been postulated that this depends largely on racial experience with the disease, but whether that is the whole explanation is not clear. If resistance to tuberculosis in man is predominantly genetic it seems probable that natural selection through elimination of the more susceptible strains should take place rather soon after racial contact with the disease. There are indications that the Negro in the United States is to some extent making such an adjustment. During the same period, however, he has been experiencing changes in social environment, the effects of which can hardly be separated from biological factors.

Kallmann and Reisner[42] contend that resistance in man is genetically multifactorial; that not only is the incidence of tuberculosis in identical twins higher than in fraternal twins and much higher than in the general population but that the disease in identical twins tends to be similar in type and in time of appearance. As evidence of the hereditary character of resistance to tuberculosis they cite morbidity rates in persons of varying relationship (Table 2).

In a study based on family case histories from Williamson County, Tennessee, Puffer[53] found that parents,

siblings and children of tuberculous patients consistently experienced higher morbidity and mortality rates from tuberculosis than did the relatives of their consorts, and that this difference persisted whether or not there was exposure in the household.

In relation to tuberculosis the human host is clearly conditioned by the factors of age, sex and race. Striking changes in the distribution of the disease by age and sex have taken place in the past 50 years. They will be discussed later, along with the influence of race and its implications.

THE ENVIRONMENT. There is perhaps no infectious disease in which the etiologic role of environment is more important than in tuberculosis. There can of course be no tuberculosis without the tubercle bacillus, but aside from a part in determining the opportunities for contact between the sick and the healthy, environment has a continuing and vital influence upon the severity, course and fatality of the disease.

For epidemiological purposes environment is conveniently divided into physical, biologic and social components. Each exerts its influence in varying degree on agent and on host, and under certain conditions may determine the balance between health and disease. Often there is overlapping in the effects of these factors but they should, so far as possible, be distinguished and their influence on the course of tuberculosis evaluated.

PHYSICAL ENVIRONMENT. At the turn of the century the physical environment was considered a major factor in recovery from tuberculosis. Sanatoria were located at high altitudes in the belief that free exposure to fresh air and sunlight played important roles in destruction of tubercle bacilli, and also through a tonic effect on the patient. Subsequent experience has shown that altitude has no demonstrable effect upon the healing of tuberculous lesions, with recognition now that location of sanatoria at points convenient to the patients is far more important in recovery than is treatment in a hospital in some remote mountain area.

Direct sunlight is surely an effective bactericidal agent for tubercle bacilli *in vitro*, but there is no evidence that it can reach those embedded in the lungs of patients.

The fact that some of the highest mortality rates have been recorded in the tropics and in the arctic is in itself evidence that environmental temperature is not an important factor in the development of tuberculosis. Apparently any temperature to which man can successfully adjust himself is acceptable to this ubiquitous parasite.

Baetjer[7] has well summarized the influence of climatic factors on occurrence of tuberculosis: "There is no sound evidence at the present time that temperature, humidity or any other factors which make up climate influence the susceptibility of either man or animals to tuberculosis or alter the course of infection."

The high incidence of tuberculosis in stonecutters exposed to excessive concentrations of silica dusts has long been recognized. Gardner[33] found that in guinea pigs lesions from infection with the virtually avirulent Saranac R1 bacillus could be converted to progressive tuberculosis by continued exposure to silica dust. Silica ions are able to stimulate the growth of tubercle bacilli in cultures and in tissues. That exposure to dusts other than those containing free silica may favor tuberculous infection and disease is as yet unproved.

Biological Environment. In a disease where susceptibility to infection is general, immunity relatively short-lived and inconstant, and the balance be-

tween the invasive power of the parasite and the resistance of the host easily disturbed, biological factors assume unusual importance.

Until well past the beginning of the present century the wide prevalence of open tuberculosis in most parts of the country was sufficient to account for continuation of the disease. For the past 20 years or more it has become increasingly important to consider the less obvious and precise sources of infection. No doubt the chief reservoir of infection consists of patients with positive sputum who in spite of more general hospitalization and better public health supervision, continue to infect a certain number of immediate contacts.

The danger of direct contact depends upon such factors as intimacy and duration, the age of the contacts exposed, the personal hygiene of the patient and whether or not the disease is recognized and reported. Improvement in bacteriological methods has brought to light increasing numbers of patients who excrete tubercle bacilli though previously classified as sputum-negative. Shaw and Wynn-Williams[61] have recently published an article on infectivity in relation to sputum status, (Table 3). Here they clearly show that the percentage of household contacts of patients negative on smear examination but positive on culture, who react to the tuberculin test, is significantly higher than the rate for children in contact with "culture negative" patients. The morbidity rate was much higher in the contacts of the "sputum positive" group.

Healthy carriers of the tubercle bacillus, if such exist, are extremely rare and are probably not a factor in transmission of infection. Most clinicians are familiar with the former patient who years after apparent recovery, is apt to excrete tubercle bacilli every time he contracts a severe cold. Such people rarely suffer exacerbation of the disease and are probably seldom sources of infection on account of knowledge of how to limit transmission. Thoracic surgeons have been concerned about the considerable proportion of patients who continue after thoracoplasty to excrete small numbers of tubercle bacilli long after clinical recovery seems complete. There is little evidence, however, that such patients are a serious hazard to their associates.

The question is often raised whether children who commonly yield tubercle bacilli by gastric lavage during the acute, infiltrative stage of "primary" infection are of practical importance in the transmission of tuberculosis. Bluhm[8] has recently reported a study on the household contacts of over 1,000 such patients with demonstrated tubercle bacillus, compared with a group

TABLE 3.—RESULTS OF TUBERCULIN TESTING IN THREE CONTACT GROUPS AND ONE NON-CONTACT GROUP OF CHILDREN AGED 0 TO 14 YEARS, BEDFORDSHIRE, ENGLAND

Contact Group	*Total No. Tested Age 0–14*	*Reactors*		*Nonreactors*	
		Number	*Per Cent*	*Number*	*Per Cent*
Positive for tubercle bacilli (Ziehl Nielsen smear)	374	244	65.2	130	34.8
Positive for tubercle bacilli on culture	228	61	26.8	167	73.2
Negative for tubercle bacilli on culture	221	39	17.6	182	82.4
Noncontact group	709	157	22.1	552	77.9

Source: Shaw, J. B., and Wynn-Williams, N.[61]

who were negative. The number of cases of tuberculosis developing in the contacts of gastric-positive cases was no greater than among the contacts of the gastric-negative cases. Wallgren[65] stated that no proven infection by such children with primary tuberculosis is recorded. Dubos[24] has said: "It is my conviction that many aspects of the pathogenesis and epidemiology of tuberculosis will remain obscure until more is learned of the mechanisms of converting inapparent infections into active disease."

Fifty years ago the bovine tubercle bacillus was an important source of tuberculosis in man. In 1928, Möllers[48] summarized the relative frequency of morbidity from the bovine bacillus in man in various countries. He estimated that in children under 16 years, 22.2% were bovine type.

In 1948, Heaf and Rusby[38] estimated that 40% of the milch cows in England and Wales were tuberculous and that an average of 6.7% of raw market milk contained virulent tubercle bacilli. At that time there were approximately 2,000 deaths per year, mainly in children, due to tuberculosis of bovine origin. Griffith[37] has estimated that 17 to 48% of extrapulmonary tuberculosis in England is due to the bovine type of bacillus, with differences according to age. In Scotland, Ireland and Holland the proportion of tuberculosis of bovine origin has been particularly high. In general, the level of bovine tuberculosis in man is determined by the extent to which the milk of cows is consumed in the raw state.

One measure of the incidence of bovine tuberculosis in man is the ratio of extrapulmonary to pulmonary tuberculosis. In the United States that ratio is about 7%, compared with about 20% in Great Britain. Since 1910 the mortality from extrapulmonary tuberculosis in the United States has declined more rapidly than has the rate from pulmonary tuberculosis. About 1916, a concerted program was launched for the eradication of tuberculosis in dairy cattle; as a result less than one half of one per cent of dairy herds include cattle reacting to the tuberculin test. Pasteurization of milk began about the same time and it is estimated that 90 to 95% is now pasteurized before consumption.

Nutrition. Of the biological factors involved in the etiology of tuberculosis, nutrition is perhaps the most important. Even before the discovery of the tubercle bacillus the value of a liberal diet in the treatment of tuberculosis was generally recognized. And, conversely, the association of phthisis with malnutrition was apparent. Complete proof of the role of malnutrition in tuberculosis is still lacking but the evidence is convincing.

Practically all studies on the subject in different parts of the world have shown that tuberculosis mortality rates are the lowest where the economic status is highest, and *vice versa*. Almost without exception the inverse correlation between descending income and ascending mortality is striking. Typical studies of this type are those by Green[36], Bradbury[13] and Wolff[67a]. Although the effects of malnutrition cannot be separated from the results of overcrowding, low income and hard physical work, it is certainly a major factor.

More specific is the evidence of a study[28] in Denmark during World War I. At the beginning of the war Denmark was one of the principal dairying and milk-producing countries of Europe. On account of the great demand for these products by belligerent countries, the greater part of the Danish output was exported during the first three years of the war. Prices rose to the extent that lower income groups

in Denmark suffered a serious food shortage. During this period the tuberculosis mortality rose from 138 per 100,000 in 1914 to 176 in 1917. At that time the Allied blockade and Germany's unrestricted submarine warfare shut off most of Denmark's export trade and the Danes were forced to eat their food products themselves. Food prices fell and food consumption of the general population returned to a liberal level. The tuberculosis mortality in 1918 fell to 138 and continued to decline thereafter. This quick response to improved nutrition is especially convincing because the improved nutrition was the only variable of significance, and it came at a time when the tuberculosis mortality was still rising in the combatant countries.

Another striking example of the apparent effect of malnutrition on tuberculosis occurred in Germany shortly after the war. The tuberculosis mortality rate which in 1918 was 230 per 100,000 had fallen to 137 in 1921. The financial crisis of 1922 led to inflation, and undernutrition reappeared though less severe than during the war. By the end of 1923, the currency stabilized, nutrition improved and the mortality rate promptly fell again.

During World War II, one of the most pertinent examples of the correlation of malnutrition with increased tuberculosis mortality was the observation of Forel[30] and of Gerard, Plauchu and Viallier[34] of the same year, that persons in mental hospitals and prisons showed an increase in tuberculosis, an increase in the proportion of fulminating, rapidly progressive disease, and that the only change which could have been responsible was the reduction in rations. Their observations were confirmed by Bourgeois, Vie and Bellin[12] who reported in a large mental hospital in Paris a sharp rise in tuberculosis morbidity and mortality coinciding with substantial food restriction and weight loss.

It would appear that there are well authenticated instances where malnutrition was the only probable cause of a rise in tuberculosis morbidity and mortality, though in most instances it is one of several associated possible causes. There are also indications that malnutrition becomes operative as an etiological factor in tuberculosis only when a critical level is reached. On the other hand, it is recognized that optimum nutrition gives no absolute protection against tuberculosis, if other circumstances are unfavorable.

The question of what specific nutrients, if any, are responsible for the effect of nutrition is still under discussion. Schröder and Kaufmann[60] concluded that the tuberculosis rates were associated mainly with fluctuations in animal protein. Tuberculosis mortality rates in England and Wales from 1938 to 1946 were studied in conjunction with intake of first class protein during that period. In the words of Dubos and Dubos[25] they showed "an astonishing inverse correlation between the consumption of animal protein and tuberculosis mortality."

A few well controlled studies on the effect of low, medium and high protein on the course of tuberculosis in rats have been inconclusive. Similar studies on variations in fat and carbohydrate in the diet of rats showed no specific effects.

Deficiency of vitamin C in the body fluids of tuberculous patients is generally recognized[62] and vitamin C in large doses has often been used therapeutically, especially for patients with intestinal tuberculosis.

If nutrition is an important factor in resistance to tuberculosis it seems pertinent to ask: how does it act?

1. Does malnutrition increase the probability that (other things being

equal) a tuberculin-negative person will acquire infection and become tuberculin-positive?

2. Does malnutrition increase the probability that an infected person will develop active tuberculosis?

3. Does malnutrition exert an adverse effect on the resistance of a person suffering from active tuberculosis?

Everything we know about the pathogenesis of tuberculosis indicates that the answer to question 1 is a clear no. Such evidence as we have from preventive medicine suggests that the answer to question 2 is yes. All the evidence from clinical experience definitely says that the answer to question 3 is also yes.

In conclusion it may be said that while definite malnutrition plays a significant role in susceptibility to tuberculosis in man, nutrition is only one of the factors contributing to normal resistance.

Social Environment. Over the years the truth of Osler's remark that "tuberculosis is a social disease with medical aspects" has become increasingly evident. Ever since that classical statement was made, the course of tuberculosis has consistently fallen or risen in inverse ratio to the standard of living. Not only does the morbidity and mortality of tuberculosis vary from country to country and from decade to decade, but within a single country variations between states and between urban and rural areas are of a magnitude of the greatest significance to the epidemiologist.

Of all the environmental factors that influence tuberculosis, that of the social environment is the most difficult to define and evaluate. In the first place many social factors are highly dependent on each other. Poverty almost inevitably affects housing and nutrition, and occupation may determine all three. How to measure their separate effects on the development of a disease of multifactorial causation such as tuberculosis, is a major problem for both epidemiologist and sociologist.

There is considerable evidence that industrialization has been an important factor in determining levels of tuberculosis. In England the peak of mortality was apparently reached early in the nineteenth century, at approximately the time of the industrial revolution. Great numbers of people were moving from rural districts to the towns. The shortage of housing was acute, overcrowding must have been extreme, the food supply was inadequate, the work was exhausting and the hours of labor were long. At the same time people from areas where tuberculosis was at a relatively low level were brought into close contact with heavily infected groups under the most unfavorable conditions.

A number of attempts have been made in industrial centers to evaluate the influence of such factors. In 1931, Bradbury[13] made an intensive study of conditions in Jarrow and Blaydon in Tyneside, England. He showed that overcrowding and tuberculosis are statistically related, in that tuberculosis increases with the degree of overcrowding. Poverty, however, was judged more important than overcrowding. In these towns, overcrowding tended to precede tuberculosis and to be associated with multiple cases. Poverty showed a high association with tuberculosis and appeared to be the cause rather than the result of it.

Bradbury considered that undernourishment was a true predisposing cause of tuberculosis and that shortage of milk appeared to be a significant factor. He found four times as much undernourishment in the nontuberculous population of Harrow as in that of Blaydon and the tuberculosis death

rate in the former town was double that in the latter.

Wolff[67b] has shown that tuberculosis mortality rates are on the whole lower in industrialized countries and that the rate of decline has been more rapid in industrial than in agrarian countries. That probably was not true in the early stages of industrialization, and in the United States today the mortality is much lower in the agricultural states. It seems probable that in Wolff's study the lower rates were due to higher standards of living made possible by industrialization.

The close association between economic status and tuberculosis mortality has been demonstrated by many investigators in various places. Among the early studies is one by Charles V. Chapin[17] in Providence, Rhode Island. He found that among the nontaxpayers of Providence in 1865 the death rate from pulmonary tuberculosis was 431, compared with 140 in taxpayers.

One of the best detailed studies of the relation of economic status to tuberculosis mortality is that of Terris[64] in Cleveland, Ohio. Using the 1940 census data he was able to divide the population into four economic levels and to compute the tuberculosis mortality for each group. The first three decades of life gave a moderate difference in mortality rates according to economic level for both males and females. Above the age of 30, there was little difference in mortality rates for females according to economic status but a great difference in the mortality for males. This excess among males was associated with the conditions of employment, 50% of the employed males being in the lowest economic bracket. Physical overstrain seemed the most probable cause. Such an interpretation is consistent with the experience of Massachusetts where a slight rise of tuberculosis mortality during World War II was due entirely to an increase among males over 40.

In addition to the influence of such social and economic factors as income, nutrition, overcrowding and occupation on the level of tuberculosis, there appears to be a biological adjustment of a population to the disease. This is illustrated by the movement of tuberculosis mortality in different provinces of Sweden from 1801 to 1825[49]. At the beginning of the nineteenth century the highest rates were in and around Stockholm, and the lowest in central and northern Sweden. By 1820, the rates in the north had risen substantially. In 1916–1920 the rates were highest in northern Sweden, intermediate in the central, and lowest in the southern part of the country. By 1925 the mortality was declining in all but isolated areas where the rates had previously been low.

In Norway, where mortality rates are available since 1881, tuberculosis has followed a similar pattern, the areas where tuberculosis first became a major problem now having the lowest rates. The conclusion is that "among a population in which tuberculosis has been prevalent for several generations, an increasing resistance has gradually grown up, which has resulted in a declining death rate from the disease." In both Sweden and Norway the decline apparently began before any direct measures were taken against tuberculosis and without improvement in the standards of living or hygiene.

FACTORS DETERMINING DISTRIBUTIONS OF TUBERCULOSIS. The multiplicity of factors involved in the causation of tuberculosis has been presented, with clear indication that the relative importance of these factors varies substantially with time, environment and type of host. It remains to summarize the relationship of these factors and their probable role in observed distri-

butions of tuberculosis within a population.

Race. It appears that long, intimate association with tuberculosis is the basis of racial resistance to the disease. Rapid elimination of the more susceptible human strains, especially during early experience with the disease, is believed to have laid the foundation for races capable of meeting the tubercle bacillus on fairly equal terms, and of more than holding their own as environmental conditions improved. That such natural selection does not provide a degree of resistance sufficient to offset unfavorable living conditions is suggested by the continuing high tuberculosis morbidity and mortality among the peoples of such ancient civilizations as China, Egypt and India where phthisis seems to have antedated medical history.

That race is not of itself the sole factor in susceptibility to tuberculosis is suggested by mortality data from Charleston, South Carolina[41]. In that city from 1822 to 1848 the death rates in whites and in Negroes were practically identical and both were declining. Beginning in 1865 the tuberculosis mortality rate in Negroes increased sharply and from then until 1900 was two to three times the rate in whites. Also at the present time the tuberculosis mortality in Negroes is substantially higher in northern cities than in the rural areas of the south. In Europe the genetic background of national populations is so complex that it is difficult to make comparisons of racial susceptibility to tuberculosis. However, for the past century at least, tuberculosis mortality among the Irish and Scots has been consistently higher than among the English. Even in Massachusetts, Pope found that in second generation Irish the mortality rate was over twice that in the native born of native parents.

Heredity. The idea of heredity as carrying a constitutional predisposition to tuberculosis was abandoned long ago, after conclusive evidence of infection was generally accepted. While susceptibility to tuberculosis is not directly inherited, that does not mean that heredity is of no importance in terms of human resistance to the disease. In animals, geneticists have been able to establish strains consistently susceptible or resistant to tuberculosis and there is reason to believe that in man heredity plays an appreciable role in the pathogenesis of tuberculosis. There is no question of the importance of personal contact in the spread of tuberculosis in families but that does not preclude the possibility that inherited susceptibility may at the same time influence the level of individual resistance. The family studies of Puffer indicate significant differences between the tuberculosis attack rates in the siblings, parents and children of index cases of tuberculosis and the corresponding relatives of their consorts, even when family contact is not involved. Even though little or nothing can be done about heredity in man its potential significance in the trends of tuberculosis is not to be overlooked.

Age. The age distribution of tuberculosis is one of the principal characteristics of the mass disease. Unlike most acute infections, tuberculosis is marked by a peak in the first two or three years of life. There is no evidence of a transmitted maternal immunity. While the infection rate rises steadily from birth, the period from 5 to 10 or 12 years carries the lowest morbidity and mortality of the entire life span. A second wave of tuberculosis starts with adolescence and reaches its peak in the 20 to 29 year age group. In females this is followed by a gradual dip in mortality and a final rise in the last three decades of life. In males, however, the

rate continues to rise from age 30 years to the end of life.

The striking changes which have taken place in this pattern are illustrated in Fig. 1. No longer is tuberculosis primarily a disease of young adult life in the United States, but rather a disease of middle life, and in men of old age. While the causes of this change in age distribution are still not clear,

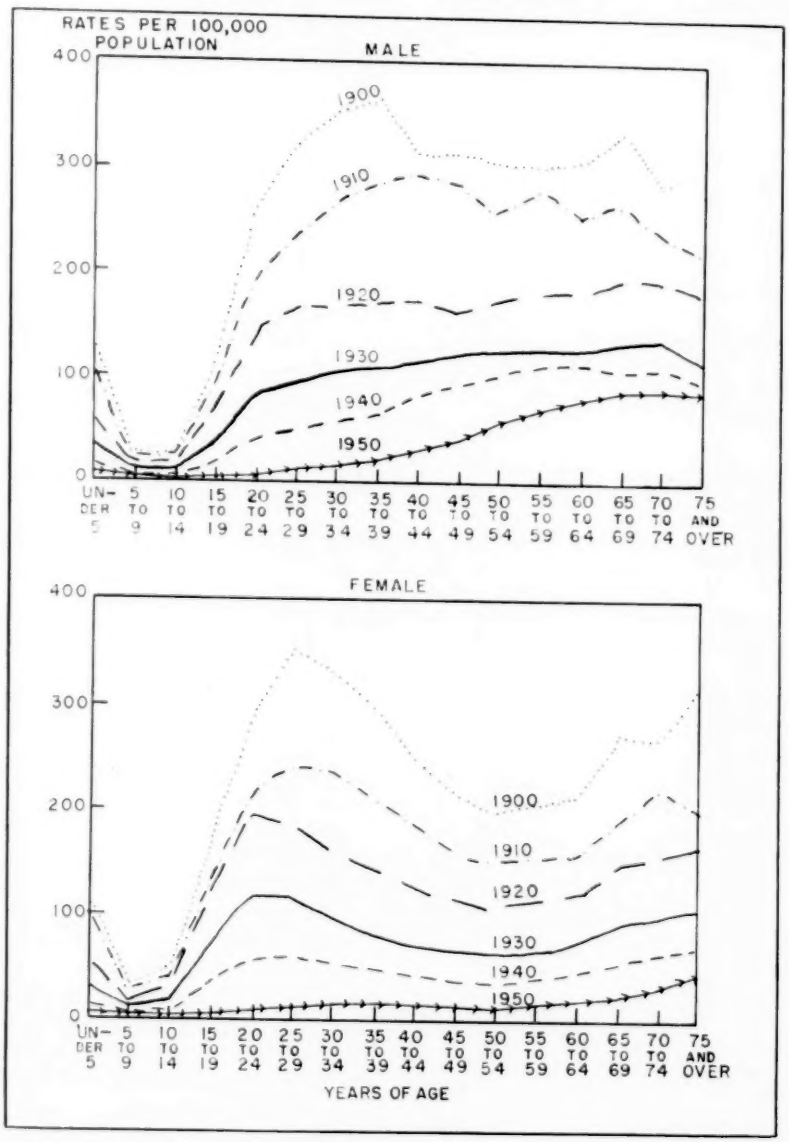

Fig. 1.—Deaths from tuberculosis per 100,000 population, by 5-year age groups and by sex, United States Registration Area, 1900, 1910, 1920, 1930, 1940, and 1950. Source: **Chadwick, H. D., and Pope, A. S.**[16]: Vital Statistics of the United States, 1950, Washington, U. S. Government Printing Office, 1953. 1950 figures.

from the epidemiological viewpoint it is believed to reflect the adjustment of man to the disease. The tuberculin test shows a remarkable decline in the level of infection in the past 30 years as well as postponement in the age of infection. There is much evidence that the high mortality of old age is the residual of the tuberculosis through which the older age groups have passed rather than a decline in resistance to new infection. The implications of such an interpretation on the control of tuberculosis will be discussed.

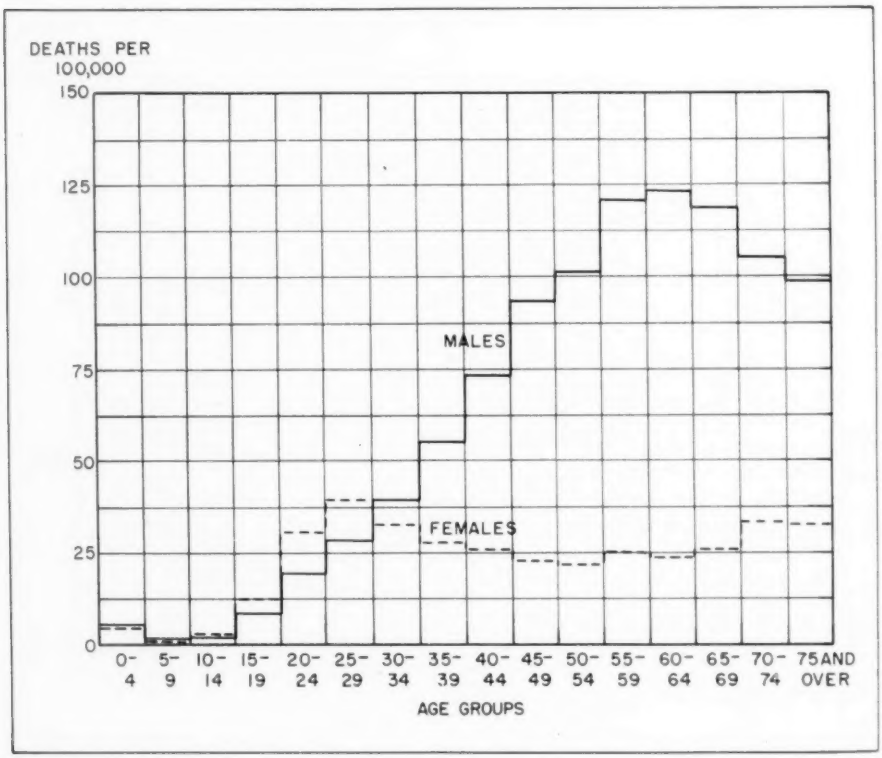

Fig. 2.—Massachusetts death rates by age and sex, from pulmonary tuberculosis, rates per 100,000 population, 1943–1947. Source: **Pope, A. S.:** Massachusetts Department of Public Health, unpublished data.

Sex. One of the greatest changes observed in tuberculosis in the past century is the much more rapid decline of the disease in women than in men. A hundred years ago the mortality rate in females was some 40% higher than in males. An excess mortality in men first became apparent in the industrial states and only in the past 15 to 20 years has the male rate exceeded the female in a few of the rural areas. For the country as a whole the female mortality now exceeds that of the male only at ages 10 to 29 years. The trends of tuberculosis mortality by age and sex for Massachusetts are shown in Fig. 2.

The causes of this remarkable change in the distribution of tuberculosis by sex are largely speculative. The more

rapid decline in females may well be associated with a decrease in average size of family during that period. Undoubtedly the improvement in living conditions has affected women more directly than men and in the change from an agricultural to an industrial economy their physical work has been more reduced. The lower level of tuberculosis in young women may be an important factor in the rapid drop of the infection rate in children in recent years.

Socio-economic Conditions. Next to the reduction in exposure to infection, and probably associated with it, there is reason to believe that improvements in the standard of living have been a major factor in the decline of tuberculosis. In the world at large the highest prevalence of the disease is found in countries where the level of economic conditions is the lowest and the greatest reduction has occurred where living standards have improved most rapidly. Poverty is often considered the basic socio-economic element causally related to tuberculosis, but poverty itself is a complex condition. Is it the overcrowding, the lack of education, the poor hygiene, the malnutrition or the exhausting occupation which is the phthisiogenic factor? The whole is so inseparably involved that it is next to impossible to separate the constituent parts. Of the socio-economic complex, nutrition is the element which seems to be most consistently associated with tuberculosis.

The profound influence of the total environment on the course of tuberculosis in a population group has been described by Dormer and his associates[21]. In a survey of some 20,000 Bantus in Natal they determined differences in incidence and type of tuberculosis in people of a uniform racial stock, differences associated with the conditions under which the native people lived and worked. Bantus living on tribal reservations showed an infection rate of 40 to 50% by adult age; the amount of active disease was remarkably low. On mission reserves, where living conditions were more crowded and there was more contact with the outside world, the incidence of progressive tuberculosis was approximately three times as high. In semi-urban areas, where the natives were crowded into shanties and nutrition was less adequate, the incidence of tuberculosis was still greater and the proportion of progressive primary and reinfection type tuberculosis was much higher. In urban areas the same type of people, living in grossly overcrowded quarters and doing heavy physical work on a deficient diet, had an infection rate of 70 to 80% and a morbidity rate six to eight times that of Bantus living in the native reserves.

Modern warfare is one of the imponderables in tuberculosis control. Involving as it does entire populations of many nations, it invariably breaks down the barriers to disease which have been built up over the decades. In World War II the tuberculosis mortality in the invaded countries rose from 50 to 200%. Not only does this represent an imposing immediate loss of life but also a dissemination of infection among entire populations from the effects of which they hardly recover in a decade. Man cannot plan the control of tuberculosis with any assurance while war remains uncontrolled.

Exposure to Tubercle Bacilli. Because the tubercle bacillus is the indispensable agent of tuberculosis, the opportunities favoring exposure to infection are bound to be a determining factor in distributions of the disease. In tuberculosis, however, the potentiality of infection is limited by the resistance of the host, for of all individuals infected only a small percentage even-

tually develop progressive disease. Here, resistance of the host, influenced probably by age, environmental factors and possibly by dosage, apparently determines the outcome. Infection rates in children in certain areas where data are available have fallen over 75% in the past 25 years. Even at adult ages, where practically all individuals reacted to the tuberculin test at the turn of the century, only about 50% show infection.

A significant limiting factor in the transmission of tuberculosis is the fact that, unlike the situation in such diseases as diphtheria and measles, only a small proportion of the individuals affected become vectors of tubercle bacilli. In the great majority, as Frost[32a] has pointed out, the tuberculous focus remains walled off in the body. Only when it breaks through to the respiratory passages, or rarely to the skin, does it become a source of infection to others. Healthy carriers of the tubercle bacillus are practically unknown.

The importance of direct personal contact in the transmission of tuberculosis is emphasized by the high attack rates among the household contacts of active cases. In a series of 680 families in groups containing "index" cases, Aspin[6] recently found five times the expected number of cases of adult type tuberculosis in the household contacts.

Intercurrent Disease. It is widely believed that co-existence of other disease in patients with tuberculosis has a deleterious effect on the course of the tuberculous process. Measles, influenza, pneumonia and diabetes are most frequently cited as examples of diseases causing such antagonistic effect.

The most widely discussed and most controversial example of a presumed impact of a co-existent disease on tuberculosis was provided by the influenza epidemic of 1918. Pandemic influenza reached the east coast of the United States in September, 1918, and within 6 to 8 weeks had spread over practically the entire country, taking a toll of some 310,000 lives. The tuberculosis mortality rate for that year rose some 10 to 12%, the increase being almost entirely in males.

Medical opinion differed widely on the part that influenza had in this increase. Fishberg[29] declared flatly that tuberculous patients contracted influenza no more frequently nor succumbed to it more often than did other individuals. Amberson and Peters[1] stated that of 1,227 former patients of Loomis Sanatorium, 70 contracted influenza and 16 or 22.9% died. There was no evidence that the severity of influenza was less in tuberculous than in nontuberculous patients and the case fatality was definitely higher in the tuberculous. They believed that influenza could reactivate quiescent or inactive cases. Boisliniere[9] reported that 15 to 20% of new cases of active tuberculosis admitted to sanatoria or seen in private practice from October, 1918, to December 31, 1919, did not have tuberculosis prior to influenza but developed tuberculosis promptly thereafter.

Epidemiologic evidence suggests that the influenza of the 1918 pandemic was a distinctly different disease from that caused by present day viruses. The 1918 influenza was rather clearly responsible for the premature death of many patients with tuberculosis and presumably often reactivated latent or inactive lesions. The action of the present day influenzas is less definite.

The high incidence of tuberculosis in diabetics is well recognized and has been the subject of intensive study. Root[55a,b,56] found the morbidity of tuberculosis among diabetic children to be 13 times that of nondiabetic school children; for some 85%, the develop-

ment of progressive tuberculosis followed the onset of diabetes. In Joslin's series of 10,000 cases of diabetes, 2.5% had pulmonary tuberculosis. In Philadelphia, Boucot[11] and her associates found that 8.4% of 3,106 diabetic patients surveyed in 1946 had tuberculosis. Diabetics under 40 years had active tuberculosis three times more frequently than older diabetics; a direct correlation existed between increasing severity of diabetes and increasing prevalence of active tuberculosis. Over all, the survey revealed twice as much tuberculosis as in a comparable group of nondiabetics.

Measles is commonly considered as predisposing to tuberculosis but conclusive evidence is rarely available. A most convincing observation is that of Christensen *et al.* in southern Greenland[19]. In 1951, practically all of 4,257 people living in that area contracted measles from a sailor coming from Denmark. In April, just before the epidemic, 363 persons in one village had been fluoroscoped for tuberculosis; 54 showed signs of tuberculosis. In September, after the epidemic, 352 of the same persons were re-examined in the same manner, and by the same examiner. At that time 19 new cases of tuberculosis were discovered, and between the two examinations, 14 deaths from tuberculosis had occurred. Such an increase of cases and deaths within a period of 5 months is unusual, even in a population with the high prevalence of tuberculosis that characterizes Greenland.

EPIDEMIOLOGICAL PATTERNS IN TUBERCULOSIS. As attention turns now to the particular epidemiologic patterns manifested by tuberculosis as it attacks populations of people, the interest initially is in the clinical nature of the disease. The mass behavior of a disease commonly stands in close relation to the manner in which it affects the individual, so much so that clinical behavior is the accepted point of departure in tracing the natural history of a disease process.

As a disease, tuberculosis is characterized by a moderate degree of infectivity, a long chronic course with tendency toward recurrence, and a high ultimate fatality in the group which develops active disease. An attack confers only relative immunity and for short periods. Unlike most acute communicable diseases, infection in tuberculosis is followed by disease in only a small percentage of instances. That is, the low morbidity rate is due to the high resistance of the host to invasion. In the majority of persons the infection remains latent for the rest of life but may become activated if host resistance is sufficiently lowered.

The susceptibility of man to tuberculosis is high, and until recently infection was practically universal by adult life. Only high native resistance to the disease maintained the equilibrium between parasite and host. For nearly a hundred years, however, various factors, chiefly environmental, have tipped the balance in favor of the host in western Europe and in the United States. The equilibrium remains, however, potentially unstable, as evidenced by the effect induced by wars, famines and other major catastrophies.

While tuberculosis mortality rates in the United States have fallen over 90% in the past century there is little evidence of real change in the clinical character of the disease. Unlike the situation in scarlet fever and smallpox, the agent of tuberculosis appears to have remained practically constant. On the other hand, the host has gradually adjusted to the disease, and thanks to improving environmental conditions and possibly natural selection has steadily gained an ascendency.

The population in the United States

is relatively homogeneous in respect to infection, as indicated by the tuberculin test. That it is profoundly heterogeneous in regard to morbidity and mortality is indicated by variations in morbidity rates by states from 16 to 154 per 100,000 population, and in mortality from 5.4 to 42 per 100,000, the data being for the year 1952.

As facts accumulate about the natural history of tuberculosis it becomes more and more evident that, making allowance for the time factor, the disease follows an epidemiological pattern not unlike that of many acute infections. While host resistance appears to change slowly there is consistent evidence that it rises after prolonged contact with the disease. In fact that appears to be the basis of the differences in racial susceptibility now observed, and of the epidemic type of occurrence in groups first exposed to tuberculosis. There can be little question that the familiar variations in the pattern of morbidity and fatality of tuberculosis according to age and sex are functions of host resistance. The fact that any immunity conferred by infection is transient and relative serves to explain the frequency of relapse.

Tuberculosis is in a real sense a disease of time and place. The present status becomes understandable only when viewed as an evolutionary process. Here and there tuberculosis still occurs in the acute, epidemic form. In a large part of the world it persists as an active, implanted disease, one of the major causes of disability and death, rising or falling with economic and social conditions but generally uncontrolled. In less than one-fourth of the world population can it be considered an essentially stable mass disease, the infectious agent in relative equilibrium with its human host.

Tuberculosis in Virgin Populations. When introduced into a population which has had little or no contact with the disease, tuberculosis tends to run an acute, epidemic course. Infection spreads freely in proportion to opportunities for contact. Resistance is at a minimum and a high proportion of reactors fall ill in a short time. Primary infections tend to develop without interruption into progressive disease. The fatality rate is high. Even under such conditions, however, not all contacts become infected and not all reactors develop disease.

The clinical course of tuberculosis in a virgin population is acute. Toxicity is severe, the temperature is high and the course is measured by weeks or months rather than years. The disease tends to become generalized early and tuberculous meningitis is frequent. The age distribution is characteristic, with a large proportion of cases in children and young adults.

The classical example of this type of tuberculosis is that described by Borrel[10] among Senegalese troops in France in World War I. The disease appeared suddenly in the second year of the war. The symptoms were those of a rapid, progressive infection, without marked respiratory manifestations, but with extensive involvement of the lymphatic system. Involvement of the lungs tended to be pneumonic, and generalized tuberculosis was frequent. Death usually occurred in 3 to 4 months, even though tubercle bacilli were not always demonstrable in the sputum.

A similar situation occurred among the North American Indians[15] after they were driven out of their natural habitat and removed to reservations. Their food there was inadequate and their whole way of life was changed. Tuberculosis promptly became epidemic and death rates up to 1,000 per 100,000 were reported.

Implanted Tuberculosis. After epidemic tuberculosis has reached its peak in a population, group adjustment to the disease begins with surprising quickness. American Indians placed on reservations and experiencing acute epidemic tuberculosis had a drop in mortality from the disease within two or three decades; and by two full generations rates had fallen over 50%. The tuberculin reaction rate[5a] in children also declined, with indication that a smaller proportion of infections was progressing to active disease. Tuberculous meningitis is still more common than in the white population but the peak of mortality appears to be moving into the older age groups. Clinically, the disease tends to be more chronic than 50 years ago. Pathologically the lesions are generally less exudative and caseating, and more nearly approach the chronic fibroid phthisis of the white population.

The mechanism of this rapid decline of tuberculosis from the epidemic stage is not wholly known. The excessive fatality presumably removes quickly the more highly susceptible individuals; and even at the height of an epidemic a considerable part of the population shows a strong innate resistance. There is every reason to believe that the process of natural selection is more rapid under those conditions where differences in susceptibility are the greatest. Another probable factor is readjustment to the new environment. In this the human race has shown remarkable adaptability through the ages. Indeed it must have been a powerful factor in racial survival. It is unlikely that these are the only factors involved in the evolution of tuberculosis, for the disease is characteristically multifactorial in respect to causes of origin and course.

The course of tuberculosis in the Negro population of the United States differs in some respects from that observed among Indians. Although the Negro race probably had only limited contact with tuberculosis prior to introduction into this country, such limited records as exist indicate that their first reaction to the disease was less violent than that of the Indians. Data from Charleston, South Carolina, show that death rates of Negroes in the first half of the 19th century were lower than those a century later. In the more recent years of accurate records tuberculosis mortality rates in Negroes have averaged 3 to 4 times those of the white population; the rate of decline, however, has been nearly the same. The age distribution of deaths is now similar to that of the white population 50 years ago, with the peak of mortality in early adult life. Mortality from meningitis and generalized tuberculosis is still disproportionately high.

The most characteristic features of tuberculosis in Negroes today are its pathological type and its acute clinical course. Dissemination through the blood stream and the lymphatics is relatively frequent; and exudative, caseating lesions are common. The clinical course of the disease is only about one-third to one-half as long in Negroes as in whites[63].

Established Tuberculosis. In a population long in contact with tuberculosis the whole tempo of the disease is slowed down. Infection takes place at a slower rate and later in life. Tuberculous meningitis is relatively less frequent; the peak of young adult disease flattens and tends to disappear, especially in males. Clinically the disease appears less acute, and such complications as tuberculous laryngitis, pulmonary hemorrhage and intestinal tuberculosis are less frequent. Apparently host resistance in the population has caught up with the invasive capacity of the tubercle bacillus and in a

number of countries is tipping the balance in favor of the human host. This is tuberculosis in the United States.

Under such conditions evaluation of the several factors conceivably responsible for the current trend becomes increasingly difficult. Exposure to the tubercle bacillus has been decreased and on the whole postponed, but there is no evidence that human resistance to *infection* has been increased. On the other hand resistance to the *disease* tuberculosis evidently is higher and that is influenced by many such factors as age, sex, genetic constitution, nutrition, intercurrent disease, occupation, physical stress and various social and economic conditions. The over-all situation becomes increasingly complex. The temptation is to look for a single factor in search of that knowledge so essential to ultimate control. But that is scarcely tuberculosis; too many factors are involved. To hold other variables constant by partial correlation is statistically practical; a single factor is evaluated; but the result does not demonstrate what other factors may have been involved nor indicate their relative weight. As Pinner[32] has said in speaking of tuberculosis: "While unifactorial analysis of multifactorial phenomena is a correct method, unifactorial interpretation of a multifactorial problem is not permissible." A critical examination of the existing epidemic potential of tuberculosis in the United States seemingly has the greater value.

Epidemic Potential of Tuberculosis in the United States. There is substantial evidence that tuberculosis in the United States is now on the down trend of a long-time cycle. Few authentic records go back more than 100 years. There are indications that in England the phthisis death rate rose throughout the 18th century and reached a peak about 1800, when the bills of mortality ascribed approximately one-fifth of all deaths to that cause. A gradual decline in that country began early in the 19th century. In Stockholm[29], a peak mortality rate of 931 per 100,000 was reached in the decade 1821–1830. In the United States no reliable country-wide mortality data are available until about 1900. At that time the mortality from pulmonary tuberculosis is recorded at a point from which it has declined almost without interruption to the present day. In Massachusetts there has been a steady decline from a rate of 400 in 1850 to 10.4 in 1953.

Evaluation of the course of tuberculosis in human populations is based largely upon rates of infection, as indicated by the tuberculin test, reported rates of morbidity and mortality, confirmed and supplemented to some extent by pathological investigation.

TUBERCULOUS INFECTION. Early information on the prevalence of tuberculosis in man was based largely upon necropsy findings. As the material was drawn chiefly from indigent patients in the medical centers of Europe the rates of prevalence obtained were necessarily maximum. Even at the present time it is doubtful whether necropsy data are representative of the total population.

The relatively high specificity of the tuberculin test and the simplicity of its use now give a reasonably satisfactory measure of the rate of infection and its levels in the various population groups. Limitations of the method are variations in the dilution of old tuberculin or tuberculin derivative used, and differences in the type of test used. The intracutaneous test of Mantoux with standard dilutions of old tuberculin or P.P.D. (purified protein derivative) is now the generally accepted standard. A knowledge of the type of test and dilutions used are essential

to satisfactory comparison of the results of tuberculin surveys.

Infection with tubercle bacilli may take place at any time after birth and in the general population tends to progress at a steady rate through childhood and into early adult life, depending upon the frequency and intimacy of contact with the disease. Reaction rates at all ages in rural Norway and in Kent County, Michigan, are shown in Fig. 3.

Levels of tuberculin sensitivity generally parallel the morbidity and mortality rates of tuberculosis in corresponding areas. In Cattaraugus County, New York, Korns[43] in a tuberculin survey of white school children in 1930 found that among those who had spent their entire life in the rural county only 7.6% reacted, compared with 21.1% among those who had also lived elsewhere. At about the same time, in rural communities in Minnesota, Slater and Jordan obtained the following low rates in school children: 6 to 9 years, 5.8%; 10 to 14 years, 6.9%; 15 to 19 years,

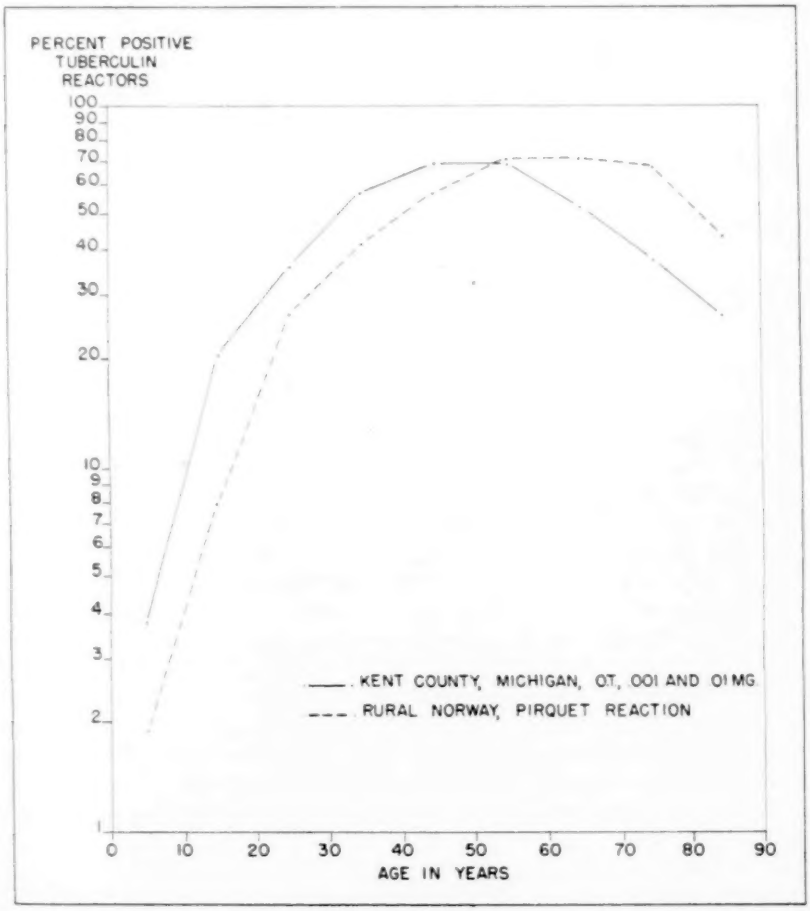

Fig. 3.—Age distribution of tuberculin reaction rates, Kent County, Michigan, and rural Norway. Source: Kent County, Michigan, **Ireland, H. D.:** Dis. Chest, **25,** 221, 1954. Rural Norway, **Myren, J.:** Acta tuberc. Scandinav., **24,** 154, 1950.

21.7%. These rates are approximately one-fourth those obtained by Aronson[5a] the following year, in white school children in Alabama and Florida and school children in cities near Boston, Massachusetts.

Reaction Rates in Contacts. The influence of household contact on infection with tuberculosis is well recognized. In Baltimore, Brailey[14] found that the risk of infection in children under 5 years of age in sputum-positive families was 18 times greater for white children and 8 times greater for colored children than in the corresponding control groups. Among Massachusetts school children in both grade and high schools, the rate of reaction to the tuberculin test was slightly over twice as high in those who gave a history of household contact with tuberculosis[16].

Infection and Sex. Among school children in the United States the rate of infection with tuberculosis appears to be a function of age. Reaction rates to the tuberculin test increase at a practically uniform rate from 5 to 18 years of age with few if any significant differences between males and females[40,54]. In adult life, reaction rates become definitely higher among males presumably on account of greater opportunities for contact with tuberculosis. This is illustrated by data from a tuberculosis control program in Massachusetts (Fig. 4).

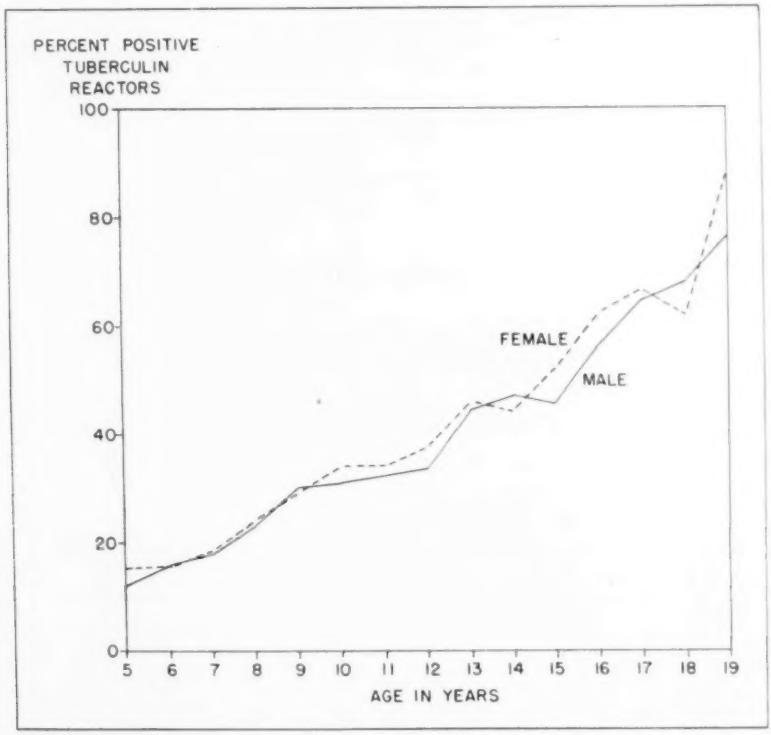

Fig. 4.—Percentage of 14,237 children aged 5 to 19 years inclusive, who reacted positively to tuberculin test, by age and by sex, Massachusetts city of 100,000, 1931. Source: **Chadwick, H. D., and Pope, A. S.**[16]

Although death rates from tubercu-

losis in this country are approximately three times as high in the colored as in the white population there is surprisingly little difference in infection rates, as indicated by the tuberculin test. In rural areas of Alabama, Florida and South Carolina, Aronson[5a] found the reaction rates essentially the same in colored and white groups tested. He noted, however, that Negroes were more sensitive to old tuberculin than whites, both qualitatively and quantitatively. In Philadelphia the reaction rates were slightly higher in colored than white. In rural Alabama, Graham *et al.*[35] found the reaction rates with 1.0 mg. O.T. slightly higher in white than in colored, but with 0.01 mg. the rates were consistently higher in Negroes and reactions were uniformly more severe. The apparent discrepancy of a high mortality associated with an average incidence of infection would seem to be explained by the relatively short, fulminating course of the disease in the Negro race.

TABLE 4.—TUBERCULIN REACTION RATES IN HIGH SCHOOL STUDENTS, PHILADELPHIA, 1929, 1939 AND 1949
RATE OF CONVERSION TO FIRST TEST O.T.

Year	*Per Cent Reaction*	*Rate per 100 Person Years*
1929	84.	5.3
1939	43.	2.7
1949	16.	1.0

Source: Teague, R. E.[63]

A serious lack in knowledge concerning trends of infection with tuberculosis is a paucity of comparable data on tuberculin reactions in the different age groups over the years. One of the few significant studies is that of Teague[63] at the Henry Phipps Institute. In three Philadelphia high schools, using a standard old tuberculin, tests on pupils at the age of 16 years were made at three 10-year intervals, Table 4.

Among grade school children in Massachusetts the reaction rate dropped from 28% in 1924 to 14% in 1939 and to 2% in 1955. If these figures are representative there certainly is no lag in the rate of decline of infection compared with the mortality rate in this country.

Morbidity. Morbidity in tuberculosis is usually measured by one of three types of data: reported cases per annum (incidence), cumulative living cases recorded in case registers (prevalence) or cases discovered by mass roentgenographic surveys of population groups. Each method is subject to error. Case reporting in tuberculosis is notoriously incomplete; it is estimated that 15 to 20% of all cases in the United States are still first reported on the death certificate. As recently as 1945, nineteen states reported less than 2 cases of tuberculosis per annual death and in 1952 the ratio of cases to deaths varied from 2.9 to 10.7 in the various states. Morbidity based on case registers is of course subject to all the errors, diagnosis and reporting, plus failure in many places to make corrections for death, recovery or movements of the population. Community roentgenographic services as a rule do not include the population under 15 and rarely cover more than two-thirds of the adult population. Also, the group with the highest prevalence of tuberculosis, males over 50, are least likely to come in for examination. Because cases reported as "active" are often classified according to roentgenographic appearance the ratio of cases to deaths tends

to be high in areas where a large proportion of cases are discovered by survey methods.

In a study of cases known to health departments, Anderson and his associates[2] found in survey areas a reported morbidity of 474 per 100,000, compared with a rate of 370 in other areas. At the same time these authors estimated the total of known significant cases in the United States as between 450,000 and 500,000 and of known active cases as 225,000 to 250,000. In 1952, totals of 109,837 new cases and 24,195 deaths were reported in continental United States.

In addition to the ratio between reported cases and deaths, the ratio of living cases in the community to annual deaths has often been used as a measure of the magnitude of the tuberculosis problem. In 1917, Armstrong[1] and his associates carried out a house-to-house survey in Framingham, Massachusetts. Diagnosis was based on history and physical examination, supple-

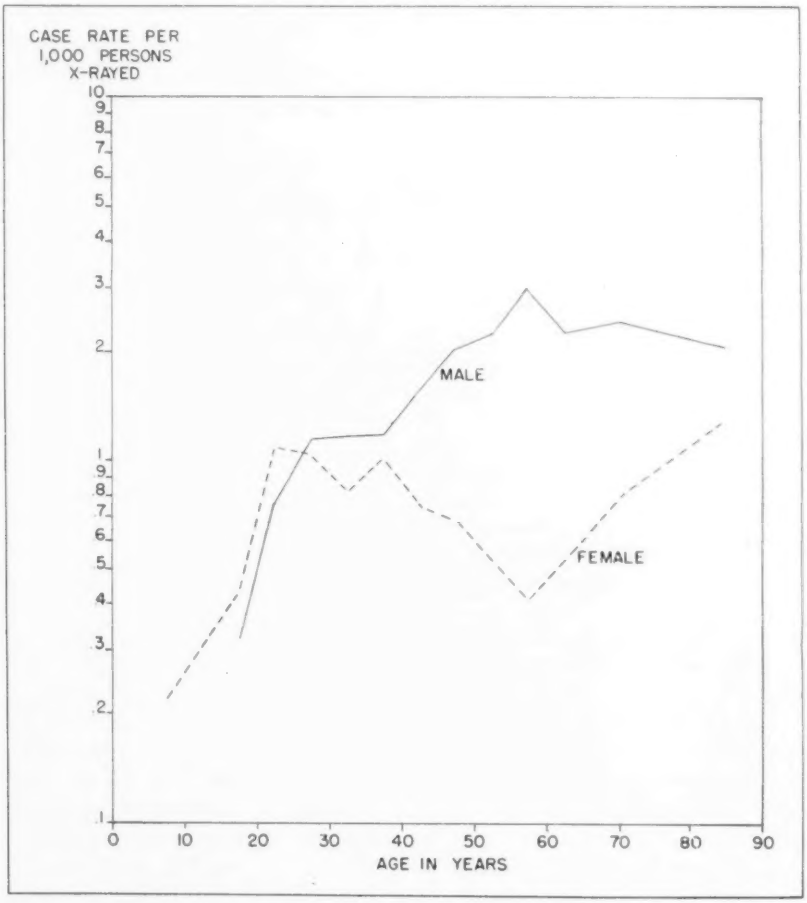

Fig. 5.—Cases of active tuberculosis discovered, and rate per 1,000 persons roentgenographed, by age and sex, Los Angeles County, 1950. Source: **Drolet, G. J.:** Los Angeles County-wide Chest *X*-ray Survey, Los Angeles, Tuberculosis Control Foundation, 1953.

mented by laboratory tests. As a result of this careful combing of an entire community a ratio was established of 9 living cases to each annual death in the town.

Roentgenographic surveys have shown that the Framingham ratio was too low and, what is more important, that the prevalence of tuberculosis varies substantially according to such

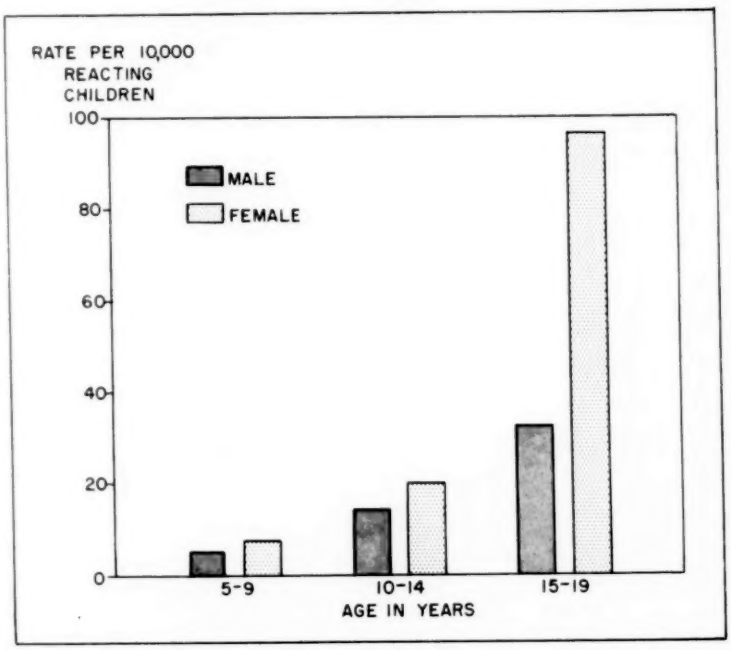

Fig. 6—Children aged 5 to 19 years inclusive, who developed tuberculosis, per 10,000 reactors to the tuberculin test, by 5-year age groups and by sex, seventeen Massachusetts cities, 1925–1930. Source: **Chadwick, H. D.**, and **Pope, A. S.**:[16]

factors as age, sex, race and economic condition of the population and the survival time of patients. Specific rates of prevalence provide a more accurate measure for epidemiological purposes.

On the basis of reported cases, the incidence rate of tuberculosis in the United States in 1952 was approximately 72 per 100,000. This is slightly less than one-half of the prevalence of active cases, as estimated by Anderson *et al.*[2]. A true "attack rate," as the term is used in acute communicable disease, cannot be computed in tuberculosis because of the long duration of the disease, 2 to 3 years, and the fact that about one-half of cases are first reported within 6 months of death.

Ideally the morbidity rate in tuberculosis should be measured in specific population groups in terms of age, sex and environment. Unfortunately few such studies have been made. Childress[18] in 1944 reported the development of tuberculosis in a group of employees at Grasslands Hospital. Of this group of 1,272 healthy people from 15 to 30 years of age, 2.2% developed tuberculosis in the course of 22 months.

The essential difference in the curves of morbidity of males and females is clearly indicated by data from chest

roentgenographic surveys. In Los Angeles in 1950, the excess of cases among females under 20 is characteristic, a difference which is greatly exceeded by the rate among males from 25 years on, (Fig. 5).

Variations in susceptibility to tuberculosis are best measured in terms of morbidity by age and sex. In several hundred thousand school children tested in Massachusetts the tuberculin reaction rate was essentially equal in males and females from 5 to 19. The prevalence of pulmonary tuberculosis, however, was significantly higher among girls, especially in the 15 to 19 year age group. On the average, tuberculosis in the girls developed some 3 years earlier than in boys. The morbidity rates among infected children are given in Fig. 6.

For comparison with incidence rates, as determined by community surveys, it is interesting to examine prevalence rates based on reported cases in a State Health Department. Such rates for up-

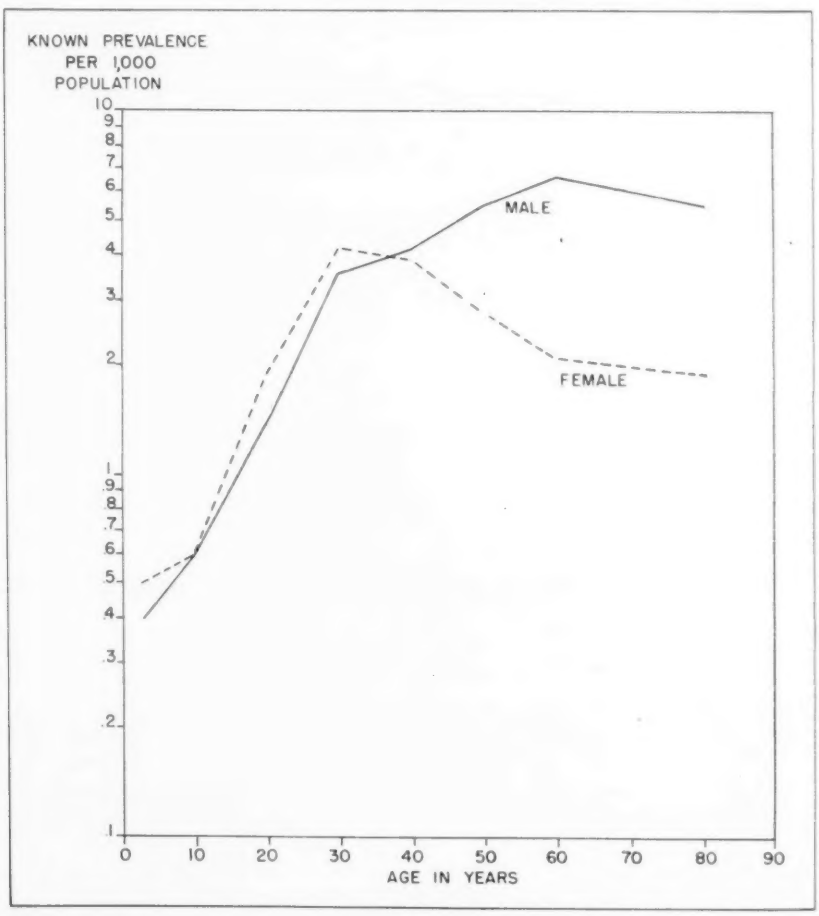

Fig. 7.—Prevalence of total registered cases, all forms of tuberculosis, New York State (exclusive of New York City), by age and sex, rate per 1,000, 1952. Source: **Wirth, H. E.,** and **Locke, B. Z.**. Health News, **30,** 14, 1953.

state New York are given in Fig. 7. Aside from the expected difference in levels of incidence the pattern is essentially the same.

The age distribution of morbidity appears to be a function of the level of tuberculosis in the country or race. Where the population has had relatively short experience with tuberculosis, the disease tends to concentrate in infancy and in early adult life. After parasite and host have become adjusted to each other, infection takes place more slowly and later in life and morbidity and mortality are relatively high in the middle and old age groups. This tendency is indicated in Fig. 8.

It is recognized that the danger of developing progressive tuberculosis is greater during the first year or two after conversion of the tuberculin test than at any other period. In connection with the increasing age at which infection is now taking place, it has been postulated that conversion in adolescence or young adult life carries a hazard that may offset the advantages of postponing or avoiding infection. Few dependable data are available on this question but a detailed follow-up

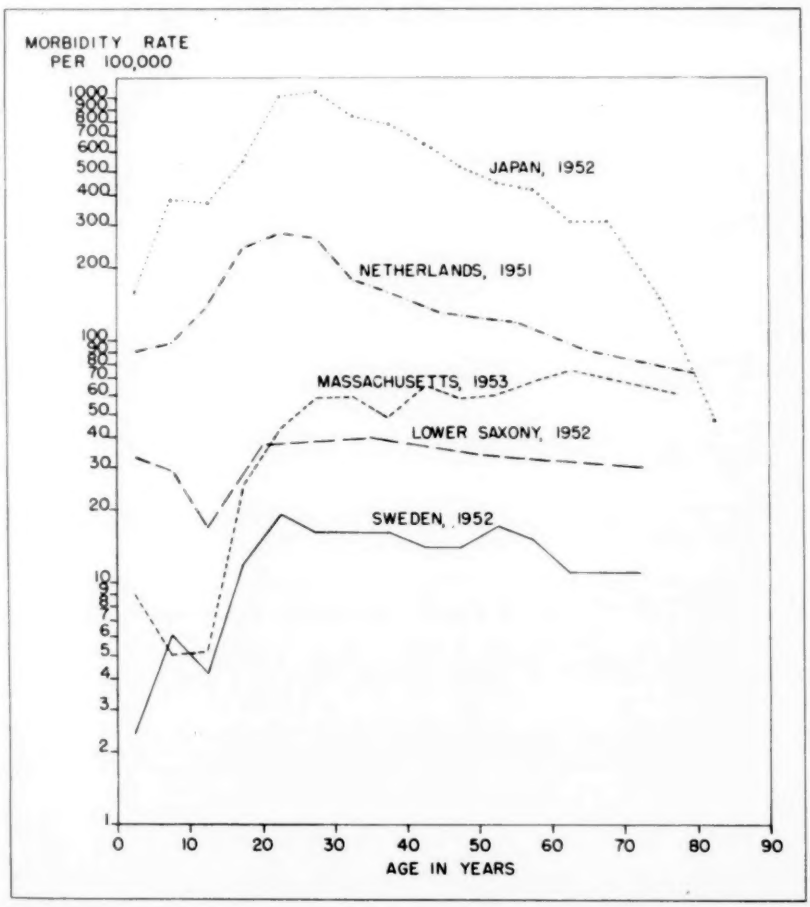

Fig. 8.—Tuberculosis morbidity rate per 100,000 population in several countries. (Active tuberculosis, all forms, registered cases). Source: **Shigematsu, I.:** Unpublished data.

of children in Copenhagen by Holm[39] following inversion (conversion) of the tuberculin test indicates that the risk of developing tuberculosis is actually greater when conversion takes place under 7 than when it occurs between 7 and 14 years, (Table 5).

Trends of Morbidity. From 1910, when the reporting of tuberculosis had become fairly uniform in the United States, to 1940, the trends of reported morbidity and mortality were essentially parallel. From 1940 to 1947, however, there was an increase of some 83,000 in the total number of cases reported[27]. In the face of a decline of 27% in the death rate for the same period, such a situation properly called for explanation. While there is no question of the implications of such an apparent increase in cases from the standpoint of tuberculosis control, it seems pertinent to examine some possible causes of the phenomenon.

In the first place, the country was at war during the greater part of this period. Over 12 million inductees into the armed forces received roentgenological examination of the chest and although the proportion found to have tuberculosis was small the number rejected was not inconsiderable.

Soon after the beginning of this period mass roentgenographic screening of civilian groups got underway, a case finding procedure stimulated by grants to states by the Public Health Service. Although the number of cases found by this means was at first small, it was estimated that in 1952 it amounted to about one-fourth of the total.

Of suggestively greater significance is the impact of these case-finding procedures on the practice of private physicians. Not only did the more accurate methods lead to the discovery of more cases but, as Sartwell has pointed out[59], the practice of establishing the diag-

TABLE 5.—INCIDENCE OF POSITIVE ROENTGENOGRAPHIC FINDINGS AFTER HISTORY OF INVERSION TO TUBERCULIN, COPENHAGEN, 1936–1941

A. First Examination at Time of Inversion

Inverters	*Total*	*Positive Roentgenographic Findings*		*Tuberculosis Bacilli Demonstrated*	
		Number	*% of Total*	*Number*	*% of Total*
Total	2,298	437	19.0	176	7.7
1–6 years	288	93	32.3	38	13.2
7–14 years	732	163	22.3	59	8.1
Adults, male	695	102	14.7	42	6.0
Adults, female	583	79	13.6	37	6.3

B. Subsequent Findings after Several Years Follow up

Inverters	*Positive Roentgenographic Findings*		*Genuine Tuberculosis*		
	Number	*% of Total*	*Number*	*% of Total*	*% of Those with Positive Roent. Find.*
Total	492	21.4	81	3.5	16.5
1–6 years	96	33.3	3	1.0	3.1
7–14 years	171	23.4	10	1.4	5.6
Adults, male	125	18.0	35	5.0	28.0
Adults, female	100	17.2	33	5.7	33.0

Source: After Holm, J.[39]

nosis on the basis of roentgenographic findings rather than on clinical examination led to the reporting of a higher proportion of cases examined. He also notes estimates by the U. S. Public Health Service that in 1952 only 55% of the total cases reported were classified as active or probably active. The reporting of suspected as well as confirmed active cases makes for more effective screening of the population for tuberculosis, but it may also result in substantial changes in the trend of reported morbidity, (Fig. 9).

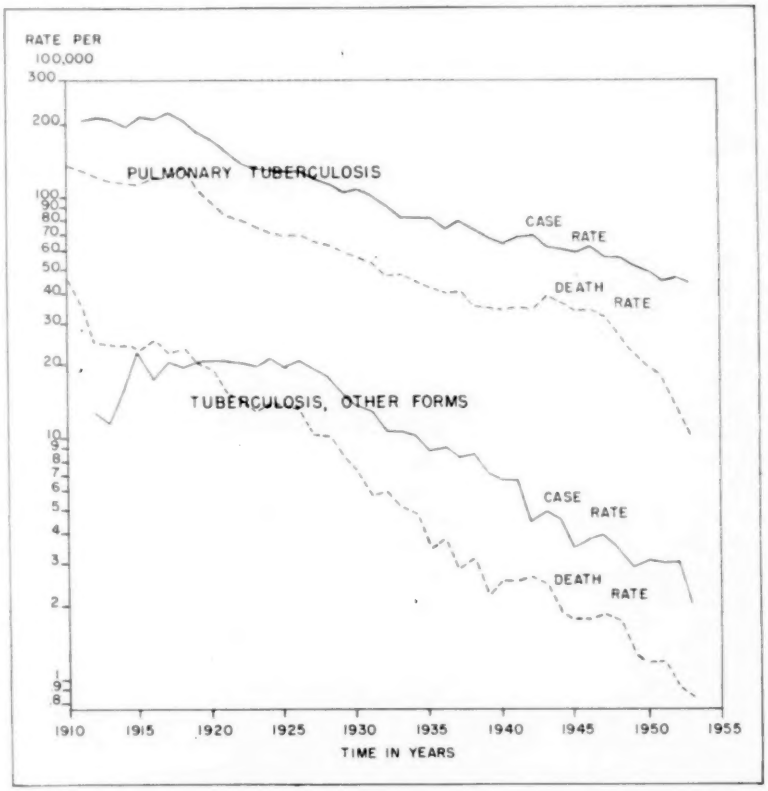

Fig. 9.—Case rates and death rates, pulmonary tuberculosis and tuberculosis other forms, rates per 100,000 population, Massachusetts, 1910–1953. Source: **Pope, A. S.:** Massachusetts Department of Public Health, unpublished data.

It is of interest to note that in the diagram by Edwards and Drolet[27] of morbidity and mortality trends, by divisions of the country, that the greatest increase in morbidity is in those areas where the ratio of reported cases to deaths has until recent years been the lowest. Variations in the practice of reporting and the type of case considered reportable have been so great that in 1949 the Director of the Division of Tuberculosis of the Public Health Service appointed a committee representative of the states to study the whole question of reporting of tuberculosis and to make recommenda-

tions. Their report has now been completed and should lead to more uniform practice.

The morbidity rate, insofar as it represents active disease, is the best measure of significant tuberculosis in the community because it stands for potential sources of infection. That being the case it should correlate with the rate of infection, as indicated by the tuberculin test. In general such a correlation seems to exist. Infection tends to occur earlier and reaction rates are higher in large cities than in rural areas, in children exposed to tuberculosis in the household than in the general population. The level of reaction is closely associated with that of mortality from tuberculosis. That being the case, what is the explanation of an increase of some 30% in morbidity accompanying a drop of the order of 60 to 75% in the infection rate, as already noted?

Admittedly the data on tuberculin-reaction rates are few and are based chiefly on groups of children but they are generally consistent.

FATALITY. Fatality rates in tuberculosis are more difficult to obtain and less reliable than any of the other standard measurements of the disease. On account of the long duration and the tendency to remission it is often difficult to associate death with the original report of the disease or to be certain whether a given patient ultimately dies of tuberculosis or of some other cause. Prior to the use of antibiotics and chemotherapy it was estimated that about one-half of patients having active tuberculosis eventually died of this disease. Ideally fatality in tuberculosis should be determined by following a substantial group of patients from the onset of the disease to death from whatever cause. One of the few attempts to collect fatality data of that type was reported by Teague in 1952[63]. In a group of patients followed from 1926–1945 in the clinic of the Henry Phipps Institute, Philadelphia, the average longevity was 5.9 years. For whites it was 11.2 years and for Negroes 3.5 years. Most striking was the mean survival time in relation to the stage of disease at the beginning of observation: Minimum 25.1 years, moderately advanced 9.9 years and far advanced 1.1 years.

The percentage of deaths within stated periods, as 5 or 10 years after onset, is often used as a substitute. Using data on 8,000 patients from Trudeau Sanatorium, Sampson[58] calculated death rates for periods of 6 years after onset. During the first four decades of the nineteenth century the rates in these groups dropped from 25% to just below 10%. It should be kept in mind that Trudeau Sanatorium patients were selected on the basis of their prognosis and that on the whole they came from the higher social and economic groups. Practically all such studies show an accelerating rate of decline in fatality for 5 to 10 years after the completion of treatment but a definite residual for longer periods.

Since the adoption of antibiotic and chemotherapy there is substantial evidence of a decline in fatality rates in sanatorium patients during the period of treatment. In a group of state and county hospitals the fatality rate which had averaged about 30% prior to 1948 had dropped to 10.8% in 1952. Such a decline may in part represent a postponement of death rather than arrest of the disease.

MORTALITY. Because deaths from tuberculosis are as a rule more uniformly and more completely reported than cases, mortality rates have generally been used as the most accurate measure of the prevalence and severity of the disease. In view of the recent decline in the fatality of tuberculosis the mortality rate becomes a less adequate

index of prevalence but is probably still the most reliable indication of the relative frequency of tuberculosis in population groups.

A notable indication of the decline of tuberculosis mortality in the United States is the practically straight line trend from 1900 to 1947; in several individual states for which data are available, this trend extends back to about 1870. For the first half of this period hospitalization and other public health procedures could hardly have had any appreciable effect on tuberculosis mortality as a whole, and the steady decline would seem to be part of a long-time trend which has been variously ascribed to a biological cycle in the disease and to increasing host resistance, as influenced by natural selection and by marked improvement in standards of living.

The accelerated decline in mortality from 1947 to 1953 may in part be comparable to the drop following the first World War and the influenza epidemic of 1918. This period also marks the beginning of effective chemotherapy and the expanding use of thoracic surgery. Case finding has certainly been improved by the examination of some 20 million people in mass roentgenographic surveys, and the increased hospitalization and supervision must have further reduced foci of infection. In short, public health methods seem to be gaining prominence among major determinants in the trend of tuberculosis.

The mortality rate is still the best single indication of the pattern of distribution of tuberculosis. In the United States for at least 50 years the rates have been consistently higher in cities than in country districts. In 1950, the urban rate, based on 59% of the total population, was 26.5 per 100,000 population, compared with a rate of 16.6 in rural areas. In Massachusetts the tuberculosis mortality rate of Boston is two and one-half times the rate in the rest of the state.

The tendency for tuberculosis to concentrate in cities has been well expressed by Pagel, Simmonds and Macdonald[51]. "On the whole tuberculosis is associated with urbanization rather than with country life. There is everywhere an increasing tendency to urbanization. Young adults tend to migrate to towns in search of occupation and thus provide virgin soil for the bacillus. When we compare industrial districts and agricultural sections, or capital cities with the country as a whole, the unfavorable influence of the town becomes obvious."

In the United States the lowest mortality rates are consistently in the agricultural states. In 1952, Idaho reported a rate of 5.4 and Nebraska 5.7 per 100,000. States with large colored populations experienced rates above the average; and Arizona, which has for years been a mecca for patients with tuberculosis regularly tops the list.

The Influence of Age and Sex. The age distribution of mortality in tuberculosis is characteristic of the disease. Unlike mortality curves of most acute communicable diseases, the peak of tuberculosis mortality in the first year of life is followed by a sharp drop, and the lowest level within the life span is from 5 to 10 years of age. Until about 25 years ago the maximum rate was reached in young adult life, about 5 years earlier in females than in males. The subsequent flattening out of this peak and the relative increase above age 40 are among the remarkable feaures of the disease. These changes in pattern during the past 50 years are shown in Fig. 1.

Another unusual feature in the course of tuberculosis mortality is the relatively more rapid decline in females than in males during the past

100 years or so. In Massachusetts the death rate among females in 1850 was 40% higher than in males; today the rate among males is 2.8 times that for females. The same trend holds for the country as a whole, but as Lumsden and Dauer[45] have shown, at wholly different rates in different areas. In general the mortality rates in women have fallen sooner and more rapidly in the industrial states, and in some rural states have only recently reached the level of the male rates (Fig. 1).

The reasons are not wholly known for this reversal of the toll exacted by tuberculosis among males and females. Possible factors seemingly include a decrease by as much as a half in size of families; a great improvement in household living conditions, a reduction in heavy physical work formerly required of women, and that heavy manual work is now carried on almost entirely by men. In this connection it is interesting to note that the slight increase in tuberculosis mortality which occurred in some half dozen of the eastern industrial states in 1943 was almost wholly among men over 40.

The causes of this change in the age and sex distribution of tuberculosis have been the subject of much investigation and discussion. Medlar[47] on the basis of his wide experience in the pathology of tuberculosis thinks that the high mortality of older men is explained by their greater exposure to infection. In the low income groups particularly, he points out that old men much more than old women are apt to have no permanent homes but live in rooming houses and other places where they are thrown in contact with open tuberculosis.

In an intensive field study of tuberculosis in Buffalo, Terris[64] expresses the opinion that in addition to the greater opportunities for extrafamilial exposure to tuberculosis among males, a major consideration is the physical overstrain characteristic of the work of unskilled laborers in the lower economic brackets. The importance of overexertion in the prognosis of tuberculosis is recognized. Certainly in the past 50 years there has been far greater reduction in heavy physical work among women than among men. The extent to which tuberculosis has become a disease of old men is indicated by Fig. 2, based on death rates by age and sex in Massachusetts.

The great variation in mortality from tuberculosis among infected persons at different ages is illustrated by Table 6. This emphasizes the great risk of infection in the first five years of life and the subsequent peaks in young adult life and old age. On the basis of recent tuberculin surveys Rich[54] has computed age-specific mortality rates for estimated infected persons. The figures differ markedly from rates based on the general population and emphasize the relative hazards of infection at different ages. These rates highlight the extremely high fatality in the first year of life, the trough of mortality from 5 to 10 years, the peak of early adult life and the steady rise from age 50 which continues as a plateau throughout the rest of life, subject to the influence of such factors as sex, occupation and economic conditions. This behavior is perhaps one of the best measures of native resistance to tuberculosis.

Race. Substantial variations in tuberculosis mortality rates of different racial groups have long been recognized. The basis of these differences is still not wholly clear. Racial experience with the disease was long assumed to be the reason for the relatively low rates among Jews and the high rates among Negroes, but observed differences in the mortality of different groups of the same race living under different condi-

tions leaves open to question whether the observed rates are due to inherent resistance or natural selection or the influence of environment.

Pathologists state that the disease in Negroes in this country is almost uniformly of the so-called "primary" type with widespread involvement of the lymphatic system and frequent generalization. Clinically, the disease in Negroes tends to run a more acute, shorter course than in whites. Some years ago Opie[50] pointed out that the average duration of fatal disease in white patients of Philadelphia aged 20 to 30 years was about 2½ years; among Negroes attending the dispensary in Jamaica the interval was only 9 months.

In mass roentgenographic surveys of some 65,000 persons in New York City, Edwards[26] found the incidence of both active and chronic pulmonary tuberculosis to be higher in the white than in the colored population groups. However, in the entire city the death rate for the colored was 5 times that for whites.

Among household contacts of sputum-positive patients and fatal cases of tuberculosis in Williamson County, Tennessee, Puffer and associates[53] reported that the tuberculin reaction rates and the incidence of manifest tuberculosis were essentially the same in white and colored contacts, but the disease was more severe in the colored.

In the U. S. Army, where living and working conditions are certainly more nearly comparable than in civilian life, Roth[57] found over a period of 15 years mortality rates in Negroes at 99 per 100,000 compared with 24 in white soldiers. Aronson[5b] likewise reported during World War II, that Negroes made up approximately 10% of the Army population and yet contributed 43.4% of total deaths from tuberculosis.

Age Selectivity. With his characteristic epidemiological perception Wade Frost[32b] pointed out over 15 years ago the significance of the mortality curve of tuberculosis at different periods of life. As the trend during the first 5 years

TABLE 6.—MORTALITY FROM TUBERCULOSIS AT DIFFERENT AGE PERIODS IN THE GENERAL POPULATION, AND IN THE ESTIMATED INFECTED PORTION OF THE POPULATION (U. S. REGISTRATION AREA, 1940)

Age	*Total Deaths from TB*	*Estimated Per Cent Infected*	*Deaths from Tuberculosis per 100,000 Persons of Each Age Period*			*Deaths from Tuberculosis per 100,000 Estimated Infected Persons of Each Age Period*
			Male	*Female*	*Both Sexes*	*Both Sexes*
0–1	496	0.5	24.8	24.3	24.6	4,920
1–4	1,047	10	12.6	12.0	12.3	123
5–9	469	25	4.6	4.2	4.4	18
10–14	775	35	5.0	8.2	6.6	19
15–19	3,375	45	19.7	35.1	27.4	61
20–24	5,752	55	41.5	57.5	49.6	90
25–29	6,243	65	52.7	59.7	56.3	87
30–34	5,775	75	60.3	52.6	56.4	75
35–39	5,448	85	67.0	47.3	57.1	67
40–44	5,438	90	83.2	40.4	61.9	69
45–49	5,222	95	90.6	34.8	63.3	66
50–54	5,058	95	101.3	35.8	69.7	73
55–59	4,409	95	109.9	38.9	75.4	79
60–64	3,641	95	110.3	42.8	77.0	81
65–69	2,975	95	105.4	51.1	78.2	82
70–74	2,179	95	107.6	62.5	84.8	89
75 and over	2,057	95	90.3	66.8	77.8	82

Source: Rich, A. R.[54]

of life is directly opposite the curve of infection at that period, and as the other major changes in the mortality rate do not correspond to known changes in the rate of infection as indicated by exposure to tuberculosis or by the tuberculin test, it appears probable that the observed changes in the mortality rate are associated with corresponding changes in host resistance. This assumption corresponds with the known fact that in tuberculosis age and prior infection do not confer the lasting immunity which results from attack of many of the acute infections.

To measure the age selectivity of tuberculosis mortality in the parts of the life span, Frost took the death rates from tuberculosis in Massachusetts at specific ages for males and females, for the years 1880, 1890, 1900, 1910, 1920 and 1930. In that way it was possible to obtain for 1880 the specific mortality for persons born in the years 1871–1880 and to follow their mortality curves through succeeding decades of life. The method is illustrated by Fig. 10 in which the cohort of 1880 has been followed to 1950.

AGE	1880	1890	1900	1910	1920	1930	1940	1950
MALE								
0–4	760	578	309	209	108	41	10	4
5–9	43	49	31	21	24	11	2	1
10–19	126	115	90	63	49	21	6	1
20–29	444	361	283	207	149	81	36	7
30–39	378	368	296	253	164	115	55	25
40–49	364	336	253	253	175	118	86	42
50–59	366	325	267	252	171	127	95	69
60–69	475	346	304	246	172	95	108	87
70+	672	396	343	163	127	95	81	113
FEMALE								
0–4	658	593	354	162	101	27	12	2
5–9	71	82	49	45	24	13	1	2
10–19	265	213	145	92	78	37	14	3
20–29	537	393	290	207	167	92	36	16
30–39	422	372	260	189	135	73	30	18
40–49	307	307	211	153	106	53	24	12
50–59	334	234	173	130	83	47	24	13
60–69	434	295	172	118	83	56	35	15
70+	564	375	296	126	68	40	36	25

Fig. 10.—Death rates per 100,000 from tuberculosis, all forms for Massachusetts, 1880 to 1950, by age and sex, with rates for cohorts of 1880 and 1900 indicated. Source: based on data from **Frost, W. H.**: Am. J. Hyg., **30**, 91, 1939; recent data from **Pope, A. S.**: Massachusetts Department of Public Health, unpublished data.

Although in 1950 the peak of age-specific mortality for males falls in the age group 70 years and over, it should be noted that this group had previously experienced its highest mor-

tality at ages 20 to 29 years. In other words, the recent high mortality of persons of 70 years and over is the residual of an even higher risk to which the individual was exposed earlier in life. The same pattern of age distribution of mortality, with the peak at ages 20 to 29, is seen in succeeding cohorts.

The persistence of this pattern was observed by Andvord[3] who used it as a basis for projecting curves of future mortality in tuberculosis. This may be an unwarranted assumption but if the present relative age specific mortality rates persist and if infection rates continue to decline the possibilities of ultimate control of tuberculosis are definitely enhanced.

MULTIPLE FACTORS IN AN IMPROVED EPIDEMIC POTENTIAL. Once a communicable disease has been brought under control it is usually possible to judge just how and where the epidemic cycle has been interrupted. In typhoid fever, for example, environmental sanitation was the critical factor rather than the practice of immunization or the isolation of patients. Reduction of the mosquito vector and limitation of its contact with man brought the level of human malarial infection so low that in this and other countries a reservoir of infection for anopheline mosquitoes scarcely exists.

The phenomenal changes of the past 50 to 75 years in the United States rates of infection for tuberculosis, of morbidity and mortality and of age distribution of the disease, cannot be so simply explained. In substance, it appears that changes in the causative organism, its infectivity, its pathogenicity or its virulence are insufficient to have exerted any marked influence on the trend of the disease. It is possible that the average resistance of the population may have been sufficiently raised by natural elimination of the more susceptible individuals to have influenced the trends of morbidity and mortality. Downes[22] and others have shown that the survival rate of families in which tuberculosis occurred is significantly lowered.

It has been argued that the hospitalization and supervision of open cases during the past half century has substantially reduced morbidity and mortality by cutting down contact between the sick and the well. At the present level of tuberculosis in some areas, such measures could be of great practical importance. To anyone familiar with the delay and incompleteness of reporting, even at the present time, and the proportion of patients actually hospitalized, it seems that this has been only one of many factors involved. Also, it should be remembered that the present trends in tuberculosis were established long before these measures became actually effective.

Some 15 years ago it was pointed out[23] that statistically there was no evidence that pneumothorax or thoracic surgery had made any impression on tuberculosis mortality rates in the general population. In view of the relatively small proportion of patients then receiving those types of treatment, the conclusion was not surprising. Since 1945, however, the sharp drop in mortality rates, together with a corresponding fall in fatality of sanatorium patients, suggests that antibiotic management and chemotherapy are a significant factor in the decline of tuberculosis.

Finally, the least tangible but probably the most potent factor in the existing favorable trend is the general improvement in the standard of living. Greater earning power has made possible more adequate nutrition and better housing. Reduction in the average size of families has reduced overcrowding, which in turn has lessened opportunities for the spread of in-

fection. Where economic levels have continued high, tuberculosis rates have fallen; when war or famine has intervened they promptly rise. It is more than coincidence that the levels of tuberculosis throughout the world are closely related to the economic level of the populations concerned.

FUTURE DIRECTION OF CONTROL MEASURES. At the present low levels of open tuberculosis in the general population of the United States concentration of case finding on groups of high incidence becomes increasingly pertinent. It is a question how much longer the yield of total community roentgenographic survey will be sufficient to warrant the cost. As the general case prevalence falls, a focus on the household contacts of known cases, on men over 40 years of age, and on other groups with high prevalence of disease will uncover an increasing proportion of the total sources of infection, and at the same time make actual preventive measures more productive.

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BOOK REVIEWS AND NOTICES

DISEASES AFFECTING THE VULVA. BY ELIZABETH HUNT, B.A., M.D., CH.B.(LIVERPOOL), Honorary Consultant Dermatologist, South London Hospital for Women. 4th ed. Pp. 236; 47 ills., and 17 color plates. St. Louis: C. V. Mosby Co., 1954. Price, $9.00.

AT first glance the fourth edition of Hunt's "Diseases Affecting the Vulva" confounds rather than simplifies the perplexing problems of vulvar disorders. As one studies it more carefully, however, it soon becomes evident that this text clarifies and in some instances even simplifies a subject with which most physicians, even gynecologists, are only superficially familiar.

The gross and microscopic anatomy and the embryologic development of the vulva are described and clearly illustrated in three chapters, after which the following are discussed: conditions produced by local infection, disorders of the skin glands, altered skin function, degenerative changes, neoplasia, and others. Vulvar disease resulting from disorders such as those in the alimentary or genital tracts, the endocrine glands, blood disorders and acute specific infections, which are usually not considered in the differential diagnosis of local lesions, are presented in detail and it is emphasized that these are common etiologic factors.

The lesions are well illustrated with 47 black and white photographs, most of which are microscopic sections, and 17 excellent color plates of disease processes. Treatment methods for each disease are indicated and in a separate section specific treatments such as asperients, sedatives, hormones, ACTH, cortisone, and various forms of physical therapy are evaluated.

This book should be of great help to any family doctor or specialist who is frequently confronted with the problem of diagnosing and treating vulvar disease. J. W.

EINFÜHRUNG IN DIE BIOLOGISCHE REGISTRIERTECHNIK. BY DOZ. DR. H. KLENSCH, BONN. Pp. 222; 142 ills. Stuttgart: Georg Thieme Verlag, 1954 (Intercontinental Medical Book Corporation, New York, U. S. Agents). Price, $7.85.

THE author, a physiologist, has performed a valuable service to all young investigators by preparing this text on recording devices and techniques as applied in the biological sciences. The first section deals with the registration of mechanical processes, employing a variety of mechanical, optical and electrical systems. There are sections on The Recording of Aero- and Hydro-dynamic Processes; Of Temperatures and Temperature Variations; The Recording of Biological Processes; The Recording of Sound; Of the Rate of Radioactive Substances; Motion Picture Recording, and the Analytical Treatment of Data and the Methods for the Artificial Stimuluation of Biologic Objects. This book is recommended to all investigators who read German. It deserves to be translated into English. R. K.

SANDOZ ATLAS OF HAEMATOLOGY. Edited by DR. E. UNDRITZ, Sandoz Research Laboratories, under direction of PROF. E. ROTHLIN. Pp. 91; 579 ills. in full color (44 plates). Basle, Switzerland: Sandoz, Ltd., 1954. Price, $7.00 (at cost).

THIS volume contains many illustrations of the blood and bone marrow elements in health and disease. In addition, parasitic forms which appear in the blood or bone-marrow, or both, are also shown. Not the least valuable, however, are photographs of artefacts which may given rise to confusion and diagnostic errors.

In general, the color illustrations are excellent and the cell descriptions are adequate. However, the terminology employed may differ from that which the reader has learned. This may offer difficulty particularly to beginning students who are taught the standard terminology adopted by the Committee on Nomenclature of the Blood and Blood Forming Organs. Aside from the matter of nonstandard terminology, which is the bane of hematology everywhere, this volume should be a valuable laboratory aid for medical students, technicians, and clinicians. C. Z.

DIE WIRBELSÄULENVERLETZUNGEN UND IHRE AUSHEILUNG. BY PROF. DR. A. LOB. 2nd ed. Pp. 272; 149 ills. Stuttgart: Georg Thieme Verlag, 1954 (Intercontinental Medical Book Corporation, New York, U. S. Agents). Price, 18.55.

THIS is the 61st progress volume in the series, "Fortschritte auf dem Gebiete der

Röntgenstrahlen vereinigt mit Röntgenpraxis." It deals with the origin of the various types of spine injuries based on numerous pathological and anatomic preparations. There are discussed the reparative processes in the various stages of such injury. There are detailed descriptions of the various methods of examination, clinical and by Roentgen-ray, in the diagnosis of such lesions. The book is profusely illustrated with photographs and roentgenograms of outstanding excellence. Radiologists and orthopedists who read German should find this a most valuable book.

R. K.

ANGIOPATHIA DIABETICA. By PROF. DR. M. BÜRGER, Leipzig. Pp. 190; 81 ills. Stuttgart: Georg Thieme Verlag, 1954 (Intercontinental Medical Book Corporation, New York, U. S. Agents). Price, $16.40.

THIS is an excellent monograph on the increasing frequence of diabetic vascular disease and its consequences. Part 1 deals with the several types of diabetic angiopathy and its pathological implications. Part 2 discusses the effects of work upon musculature and their significance in carbohydrate metabolism in normal and diabetic individuals. Part 3 deals with the conservative management of diabetic gangrene. Workers in this field who read German will find this a valuable addition to their libraries.

R. K.

NEW BOOKS

Studies on the Nutritive Value of Bread and on the Effect of Variations in the Extraction Rate of Flour on the Growth of Undernourished Children. By E. M. WIDDOWSON and R. A. MCCANCE. Pp. 137; 32 ills. London: Her Majesty's Stationery Office, 1954. Price, $1.90.

IN 1947 and 1948 experiments were conducted in two German orphanages to determine the nutritive value of breads made from flour of high extraction value compared to those of low extraction value. The children in these institutions were somewhat beneath standard in physical development. The caloric intakes of these subjects were determined in the various bread groups; certain children were selected from each group for metabolic balance determinations. The authors remark that one of the most striking observations with the bread supplementation study was the improvement in the general condition of all the children. The extraction rate had little influence on the nutritive value of the flour. Additional investigations are reported on a milk supplement, on skeletal development, on caries incidences and on animal experiments used to complement certain observations in humans. This is a delightful and informative study, of excellent design, providing important nutritional data.

C. S.

Speciation and Variation in Asexual Fungi. By KENNETH B. RAPER (Chairman). Pp. 182; illustrated. New York: New York Academy of Sciences, 1954. Price, $3.50.

THIS number contains a series of papers on speciation and variation in asexual fungi presented before the Section of Biology and the Division of Mycology of the New York Academy of Sciences.

Paul Ehrlich Centennial. By R. J. SCHNITZER AND 12 OTHER SCIENTISTS. Ann. New York Academy of Sciences, 59, Art. 2, 1954. Pp. 136; illustrated. New York: New York Academy of Sciences, 1954. Price, $3.00.

THIS number of the Annals covers the Paul Ehrlich Centennial and presents a most interesting series of papers by men, who are leaders in their fields, on topics in which Ehrlich had his major interests. They are worth listing: Ehrlich's Influence on Chemistry and Biochemistry (Hugo Bauer); Ehrlich's Side Chain Theory (Ernest Witebsky); Ehrlich and Problems of Immunity (Bela Schick); Ehrlich and the Cancer Problem (C. P. Rhoads); The Chemotherapy of Trypanosome Infections (C. H. Browning); Chemotherapy of Spirochetal Infections (John A. Kolmer); Chemotherapy of Bacterial Infections (Robert J. Schnitzer); Drug Resistance (Harry Eagle); Vital Staining (N. Chandler Foot); Blood Morphology (Paul Reznikoff).

R. K.

Symposium on Problems of Gerontology. By F. H. BETHELL, B. F. CHOW, C. S. DAVIDSON, H. J. DEUEL, JR., D. J. INGLE, J. E. KIRK, A. I. LANSING, C. N. H. LONG, H. A. RAFSKY, AND N. W. SHOCK. Pp. 141; 27 ills. New York: National Vitamin Foundation, Inc., 1954. Price, $2.50.

THERE are presented ten papers on various aspects of the processes of aging with emphasis on physiological and biochemical factors, metabolism, vitamins, hormones, stress and nutrition.

R. K.

Atiologie, Pathogenese und Prophylaxe der Struma. By PROF. DR. MED. DR. PHIL. HANS OTTO HETTCHE. Pp. 103; 24 ills. Munich: J. F. Lehmanns Verlag, 1954. Price, DM 12.50.

A CONCISE statement of what is known of the etiology, causation and prevention of endemic goitre. Students of the subject, who read German, will find this little volume useful and accurate.

R. K.

NEW EDITIONS

SEXUAL DISORDERS IN THE MALE. By KENNETH WALKER, F.R.C.S., Emeritus Surgeon to the Genito-Urinary Department, Royal Northern Hospital, AND E. B. STRAUSS, M.A., D.M. (OXON.) HON. D.Sc. (FRANKFURT) F.R.C.P., Physician for Psychological Medicine, St. Bartholomew's Hospital, London. 4th ed. Pp. 260; 8 ills. Baltimore: The Williams & Wilkins Co., 1954. Price, $3.75.

THIS book deals primarily with the neurotic patterns which predispose to male impotence. It should afford a good psychosomatic background to urologists, who are frequently called upon as consultants in male sex dysfunctions. K. C.

Local Analgesia: Brachial Plexus. By R. R. MACINTOSH, M.A., D.M., F.R.C.S., F.F.A.R.C.S., D.A., M.D. (HON. CAUSA), BUENOS AIRES AND AIX-MARSEILLES, Nuffield Professor of Anaesthetics, University of Oxford; and WILLIAM W. MUSHIN, M.A., M.B., B.S., (LOND.), M.R.C.S., F.F.A.R.C.S., D.A., Professor of Anaesthetics, Welsh National School of Medicine, University of Wales. 3rd ed. Pp. 62; 32 ills. Edinburgh and London: E. & S. Livingstone, Ltd., 1954. Price, $3.00.

THE authors made only a very few revisions in this edition. Their discussion of local anesthetic solutions is more complete and includes not only procaine, but also amethocaine (Pontocaine), cinchocaine (Nupercaine) and lignocaine (Xylocaine).

Two of the illustrations, Figs. 12 and 19, are improved and give the reader a much better picture of the anatomy described.

This text presents the anatomy of the brachial plexus and the technique for blocking this plexus very clearly. It should be of great value to all physicians performing regional analgesia. L. K.

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